THE
AMERICAN NATURALIST

Vol. LXXXVIII March-April, 1954 No. 839

GEOGRAPHIC AND GENETIC ISOLATION IN AUSTRALIAN AMPHIBIA

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This paper deals with the existence of genetic isolation between populations of a single morphological species. It is concerned, therefore, with one aspect of the problem of the evolution of races into species.

It is generally believed that a frequent pattern of speciation in terrestrial vertebrates involves the gradual development of two or more species from geographically isolated populations of a single species. In this connection, geography is important in at least two ways. First, the isolated regions may differ in their environments, and the resulting interaction of variation and natural selection will have different end-products in the various regions. Second, the inability of geographically isolated populations to exchange genes will increase the possibility of divergence.

If geographic races are incipient species, we should expect instances of geographic isolation being associated with genetic isolation. Among the Australian amphibia there are some forms that would seem to afford excellent material for studies of this sort. Reference is made to those cases where the same morphological species is found in the moist zones of the east and south-west coasts but is entirely absent from the dry central areas. It is a likely assumption that the two populations, now disconnected, were at one time continuous. This could have occurred during some stages of the Pleistocene when central Australia received more rainfall. With the increased aridity of Recent times the central areas have become unsuitable and the species in question are now restricted to the moist areas in the east and south-west parts of the continent.

I have studied two species showing this pattern of distribution, namely, *Hyla aurea* (Lesson) and *Crinia signifera* Girard. It seems improbable that the eastern and south-western populations of these two species could have been in contact during the last 10,000 years or perhaps for an even longer

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time. During the period of isolation there has been no obvious divergence in external appearance of the two groups. There is always the possibility, nevertheless, that considerable genetic divergence, of a sort not detectable by examination of externals, has occurred. One way of testing this is to cross individuals of the two populations and obtain a crude measure of genetic divergence from a study of the normality of the F_1. Crude though it may be, this type of experiment gives precisely the data required by the student of evolution. If the F_1 is normal, we can conclude that the genomes of the parents are not so dissimilar that they cannot cooperate in the control of normal development and, unless other isolating mechanisms exist, only one species is involved. If the F_1 is abnormal we can conclude that genetic divergence has reached a considerable degree and that the two parents represent different species.

The data for each species will be presented separately, and with the discussion that pertains solely to that species. Data on a related problem, the evolution of *Crinia signifera* and *Crinia tasmaniensis* in Tasmania, are also presented.

Hyla aurea

Hyla aurea occurs from northern New South Wales south through Victoria and Tasmania and west to the eastern portion of South Australia. It is not known from the western two-thirds of South Australia nor from the northern or eastern areas of West Australia. Its occurrence in West Australia is limited to the south-west corner. The eastern and south-western populations are separated by a gap of more than 1,000 miles.

The material available for the cross fertilization experiments consisted of 1 female from Burradoo, New South Wales, 2 males from Sydney (East Lakes), New South Wales, and 4 males from Pemberton, West Australia. The female was injected with 3 *Bufo marinus* pituitaries and her eggs were used on the following day. Six sperm suspensions were made, 2 of N.S.W. and 4 of W.A. individuals. These were used to fertilize 6 groups of eggs. The techniques employed were the same as those for similar experiments on American frogs (Moore, 1946). The embryos were raised in a room in which the temperatures, although uncontrolled, were subject to small variations. The range was 17.5–19.6° C. and the mean 18.5 ± 0.3.

Development of the two control groups, N.S.W. ♀ × N.S.W. ♂ was essentially the same. The four groups of hybrids, though differing from the controls, were uniform among themselves.

There were neither morphological differences nor differences in rate of development between the N.S.W. × N.S.W. and N.S.W. × W.A. embryos during the cleavage, blastula, gastrula, neurula, and tail-bud (Stage 17) stages. Pollister's Rana stages will be used (Pollister and Moore, 1937). The only difference of any sort noticed during this period was a more flaccid vitelline membrane in the N.S.W. × W.A. than in the N.S.W. × N.S.W. embryos.

The first indisputable differences were noticed when the N.S.W. × N.S.W. embryos were four days old and in Stage 20. The N.S.W. × W.A. embryos

were retarded, having reached Stage 18 or 19. As time progressed these embryos became more abnormal. The first defects noticed were in the circulatory system. The heart beat was weak or absent, even in embryos that had reached a morphological stage in which heart beat was to be expected. Gill development was retarded and in most embryos there was never any gill circulation, even in those embryos that had a contracting heart and had reached the stage when the gills were being overgrown by the operculum. In fact, circulating blood was never observed in any part of these embryos. In some of them there were masses of red pigment in the anal region.

Other characteristic defects were small eyes, poorly developed tail fins, crooked tail myotomes, poorly developed pigmentation, over-expanded melanophores, and grooves extending from the mouth to the nostrils. When these N.S.W. × W.A. embryos were 13 days old (controls 10.8 mm. total length) all were either dead or cytolizing. In the terminal stages the ventral body wall was frequently perforated, exposing the yolk-laden endodermal cells. None of these defects were noticed in the controls and some of these were kept until they underwent metamorphosis.

The defects noticed in the N.S.W. × W.A. embryos bore a certain resemblance to those associated with haploidy in *Rana pipiens* (Porter, 1939). At the time it seemed most improbable that these embryos, which were the offspring of individuals of the same morphological species, could be haploids. This proved to be the case, however, when some of the embryos were sectioned and chromosome counts made by Betty C. Moore.

These results indicate that the New South Wales individuals belong to a different species from those in West Australia, a conclusion that would never be reached with the usual methods of taxonomy.

Hyla aurea, in keeping with most species with extensive distributions, exhibits geographic variation. The relation between this variation and the experimental results reported in this paper will now be considered. Parker (1938) recognized two subspecies, *H. a. aurea* of New South Wales and *H. a. raniformis* of Victoria, Tasmania, and West Australia. Loveridge (1950) described a third subspecies, *H. a. ulongae* from northern New South Wales. The individuals used in my experiments belong to *Hyla aurea aurea* (Sydney and Burradoo, New South Wales) and *Hyla aurea raniformis* (Pemberton, West Australia).

It should be noted that *raniformis* occurs on both sides of the gap separating the eastern and south-western populations. Unfortunately I was not able to cross eastern *Hyla aurea aurea* with eastern *Hyla aurea raniformis*. This experiment might have revealed that the genetic isolation barrier is between *H. a. aurea* and *H. a. raniformis* and not between the eastern and south-western populations. In the first instance the break would be near the Dividing Range in New South Wales and in the other at the south-central region of the continent. All that can be said at the present time is that the frogs from east-central New South Wales are a different genetic species from those in south-western Australia.

If one uses a genetic definition of a species, it is necessary to give the West Australian form a different name. It should be known as *Hyla raniformis* (Parker). The New South Wales material used in the experiments came from localities near the type locality for Lesson's "*Rana aurea*", so it seems safe to call them *Hyla aurea* (Lesson).

The question of a proper designation for the Victoria, Tasmania, western New South Wales, and eastern South Australia frogs, which are similar in external appearance to the West Australia *raniformis*, will have to await further experimentation. Until crosses between these and typical *Hyla aurea* have answered the question, perhaps it is the wiser course to give all eastern forms the name *Hyla aurea*.

It is unfortunate that these dilemmas arise. They are inevitable when two different criteria, in this case morphological and genetic, are used as a basis for recognizing taxonomic categories. We generally assume that the "morphological species" coincides with the "genetic species" but there is no reason why this should be so in every case.

Crinia signifera

Crinia signifera has a more extensive distribution than *Hyla aurea* but it is similar in being broken into two populations by the dry central areas of the continent. One population appears to have a continuous distribution from the northern part of West Australia east through the northern portion of the Northern Territory to the coast in Queensland and south through New South Wales, Victoria, Tasmania and eastern South Australia. The other population is restricted to south-west West Australia.

The various crosses made always involved females with uterine eggs and these females were collected in the Sydney, N.S.W. area. In this small species the usual method of obtaining ova by squeezing the female results in a high percentage of injured ova. A satisfactory variant of the standard technique of artificial fertilization was as follows. The female was pithed and submerged in a shallow dish of tap or pond water. The body cavity was opened and a slit made in the wall of the uterus. The ova were flushed out by forcing a gentle stream of water from a pipette into the uterus. The ova were then transferred immediately to a sperm suspension.

Individuals from these localities were used: Sydney (Killara) New South Wales; several locations between 5000 and 7000 feet on Mount Kosciusko, New South Wales; Lake St. Clair and Derwent Bridge, Tasmania; Perth and Armadale, West Australia. The two Tasmanian localities are within a few miles of one another and the same is true for the two places in West Australia.

There are minor size and color differences among *Crinia signifera* from many localities and Parker (1940) has recognized three subspecies. *C. s. montana* is an alpine form at present known only from Mt. Kosciusko, *C. s. englishi* is restricted to Tasmania, and *C. s. signifera* occupies the remainder of the range of the species. It should be added that this species shows considerable geographic variation as well as local polymorphism.

As is the case with many wide-ranging species, a careful study should reveal an abundance of "good" subspecies although the value of such an undertaking might be questioned. The number of subspecies in a widely distributed species is all too frequently a measure of the abundance of material and the diligence of the taxonomist. In the case of *Crinia signifera,* I do not feel that the recognition of Parker's three subspecies will aid in our understanding of the various populations. If *montana* and *englishi* are recognized, there is the implication that the residue, the subspecies *signifera,* is homogeneous. This is certainly not the case. In the present study it seems best not to employ trinomials. For those who are interested in the subspecies involved, however, the Sydney (Killara) New South Wales, and West Australia individuals belong to Parker's subspecies *signifera,* the Mt. Kosciusko ones to *montana,* and the Tasmanian ones to *englishi.*

Sydney, New South Wales × *Mt. Kosciusko, New South Wales.* Three crosses between individuals from these localities were made. In each the eggs of a Sydney female were fertilized with sperm of a Sydney male and a Mt. Kosciusko male. No morphological nor physiological differences between the Sydney × Sydney and Sydney × Mt. Kosciusko embryos could be detected at any stage of development. After hatching the embryos were measured with an ocular scale and the mean body lengths, in mm., for the two classes of embryos in the three experiments were as follows: first experiment, 7.5 ± 0.05 and 7.2 ± 0.07; second experiment, 7.5 ± 0.05 and 7.5 ± 0.07; third experiment, 7.4 ± 0.09 and 7.4 ± 0.10. The values for the Sydney × Sydney precede those for the Sydney × Mt. Kosciusko embryos. Since growth in length is rapid at this early stage, any size difference between the two classes of embryos could be easily detected.

New South Wales × *Tasmania.* Three crosses of this type were made. In each the eggs of a Sydney, New South Wales female were fertilized with the sperm of a Sydney male and a Tasmanian (Lake St. Clair or Derwent Bridge) male. No differences between the New South Wales × New South Wales and New South Wales × Tasmania embryos could be detected. Shortly after hatching, measurements were made and the lengths in mm. of the two classes of embryos were found to be as follows: first experiment, 7.8 ± 0.09 and 7.8 ± 0.07; second experiment, 7.8 ± 0.07 and 7.7 ± 0.09; third experiment, 7.9 ± 0.11 and 7.9 ± 0.05. The values for the New South Wales × New South Wales embryos precede those for the New South Wales × Tasmania groups.

From these experiments it was concluded that *Crinia signifera* from Mt. Kosciusko and Tasmania are fully compatible with members of this species from Sydney, New South Wales. There was no evidence of genetic differences of a magnitude that would lead to defective hybrids when genes from the various geographical regions were combined.

New South Wales × *West Australia.* Four crosses of this type were performed. In each case the eggs of a Sydney, New South Wales female were fertilized with sperm of a Sydney, New South Wales and a West Australia (Perth or Armadale) male. Only three different males from each locality

were used since in one experiment the sperm of a single male of each region were used to fertilize the eggs of two females. The results of these crosses were different from those involving only the eastern populations, and led in many cases to serious developmental defects.

In the first cross the N.S.W. × W.A. embryos were normal until Stage 12. At this stage the blastopore was abnormally large as shown by measurements of the embryo diameter and yolk-plug diameter. These values for the N.S.W. × N.S.W. embryos were 1.6 ± 0.02 and 0.8 ± 0.09 mm. respectively. Values for the N.S.W. × W.A. embryos were 1.6 ± 0.04 and 1.4 ± 0.05. Since both groups of embryos were Stage 12 (early) the differences are not due to the embryos showing different degrees of development. The differences are due to the fact that epiboly is retarded in the N.S.W. × W.A. group and the formation of a 360° blastopore finds the edge of the invaginating material only a short distance below the egg equator. These defects in gastrulation frequently resulted in exogastrulation and cytolysis. Other embryos formed nearly normal neurulae. Twenty-one percent of the N.S.W. × W.A. embryos became swimming larvae that appeared to be normal enough to reach metamorphosis, though they were not kept that long. The remainder either cytolozed in neurulae or tail-bud stages or formed abnormal larvae. The principle defects were the occurrence of large external vesicles in the posterior region of the body, abnormal eyes, extra tails, necrosis of the body wall, defective pigmentation, and retarded development.

In the second experiment the N.S.W. × W.A. embryos were more normal during the gastrulation stages but many cytolozed before reaching the hatching stage and none developed into normal tadpoles. The types of defects were retardation in rate of development, small heads, swollen and twisted bodies, absence of heart beat, shrivelled tail fins, straight intestines, and necrosis of the epidermis in the abdominal region.

The third and fourth crosses involved 2 females but only 1 male from each of the two localities. The N.S.W. × W.A. embryos were nearly normal. There were slight defects of the types found in the first two experiments but nearly all of the embryos formed healthy tadpoles. These were somewhat retarded in rate of development and were darker than the N.S.W. × N.S.W. group. Some were kept until they underwent metamorphosis into seemingly normal frogs.

Taken as a whole, these results indicate considerable incompatibility between New South Wales and West Australia *Crinia signifera.* In fact, genetic divergence has reached a point where the two populations should be recognized as separate species. The proper course, therefore, requires that one be described as a new species.

The locality given by Girard (1853) for *Crinia (Ranidella) signifera* was New Holland. A later report (1858) on the same material, but using the name *Ranidella signifera,* gives this more precise information, "This species is a native of Australia; several specimens having been collected on the southeastern coast of that continent." On the basis of this state-

ment, it seems justifiable to restrict the type locality of *Crinia signifera* to Southeast Australia. There is no name among the many synonyms of *Crinia signifera* that could be revived for the West Australian form. A new name, therefore, must be proposed.

Crinia insignifera sp. nov.

Type. The type is the male used in the first N.S.W. × W.A. cross mentioned before. It was collected at Armadale, West Australia, September 24–25, 1952. It has my field number 65 and an American Museum of Natural History number A 56365. This specimen will be sent to the Australian Museum in Sydney. The paratype has my field number 68 and the American Museum of Natural History number A 56366. It was collected at the same time and place as the type.

Description. I have not been able to find any constant morphological differences that will separate *Crinia insignifera* from *Crinia signifera. Crinia signifera* shows considerable geographic variation and local polymorphism. The type, paratype, and many other examples of *Crinia insignifera* collected in West Australia are within the range of normal variation of the *Crinia signifera* I have studied and of Parker's (1940) description. The distinction is based on the fact that individuals of these two species produce many abnormal hybrids when crossed. In this instance, a biological rather than a purely morphological description of the species category is used.

The lack of distinctive morphological characters in *Crinia insignifera* is not a serious difficulty in naming individuals. Unless further experimentation proves it otherwise, we can regard all of the frogs of south-west Australia, formerly known as *Crinia signifera,* as being *Crinia insignifera.*

Crinia tasmaniensis

On the island of Tasmania there are three species of *Crinia,* namely, *C. laevis* (Günther), *C. signifera* Girard, and *C. tasmaniensis* (Günther). *Crinia signifera* and *Crinia tasmaniensis* present a very interesting problem. The two resemble one another closely. Prior to the work of Blanchard (1929) in Tasmania, there was considerable doubt of the validity of *Crinia tasmaniensis.* Up to that time the species was known solely from the types, which had been described in 1864. Blanchard found both *Crinia tasmaniensis* and *Crinia signifera* common.

I found the two species in the same field near Derwent Bridge, Tasmania. They are strikingly similar. *Crinia tasmaniensis* has a smoother abdomen than *Crinia signifera* and the posterior part of the abdomen has a reddish coloration. *Crinia tasmaniensis* was found in wetter places than *Crinia signifera.* Blanchard speaks of *Crinia tasmaniensis* as "... strictly aquatic, ...but limited to the shallowest water...." All of the individuals of *Crinia tasmaniensis* that I collected were in a habitat like that described by Blanchard and the *Crinia signifera* were under logs in drier parts of the field.

Three crosses were made between *Crinia signifera* females from Sydney (Killara) New South Wales and *Crinia tasmaniensis* males from Derwent Bridge, Tasmania. The females were the same as those used in the cross between the New South Wales *Crinia signifera* and the Tasmanian *Crinia signifera* described earlier.

The hybrids were normal in development until the early gastrula stage (stage 10). When the controls were at the end of gastrulation, the hybrids were definitely retarded. They became increasingly abnormal with the passage of time. Some died as early larvae. All showed structural defects, the most characteristic being head reduction, small eyes, bloated bodies, and crooked tails. From their appearance, it was felt they might be haploids and chromosome counts by Betty C. Moore showed this to be the case. Eventually all died, the best when they reached a length of 10 mm.

These experiments demonstrate that *Crinia signifera* and *Crinia tasmaniensis* are different species in spite of their remarkable similarity of structure.

DISCUSSION

The experiments reported in this paper reveal a complexity of interrelation of some Australian frogs that was not apparent from a study of their external characters. *Hyla aurea,* as previously recognized, consists of *Hyla aurea* in the east and *Hyla raniformis* in the south-west. A similar situation occurs in *Crinia signifera.* Formerly considered a single species, experiments have shown that the eastern form cannot be crossed successfully with western individuals. The name *Crinia signifera* is restricted to the eastern population and a new name, *Crinia insignifera,* is proposed for the different western biological species.

These two instances of close morphological resemblance between the eastern and western species may be interpreted as indicative of recent divergence, though this is not necessarily so. It appears likely that the divergence is associated with geographic isolation. In both instances the aridity of central Australia is a barrier. It is probable that this barrier has existed since the Pleistocene. During the Pleistocene there were long periods when the entire southern part of Australia had sufficient moisture (Gentilli, 1949) for the ancestors of the *Hyla aurea-Hyla raniformis* and the *Crinia signifera-Crinia insignifera* species groups to have an unbroken trans-continental distribution.

It is tempting to suggest that the formation of the eastern and western species is associated with the Post-Pleistocene aridity of the center, which divided the populations. This is not a necessary conclusion since species with a continuous distribution are known in which the individuals of peripheral populations cannot be crossed. In *Rana pipiens,* for example, populations in the northeastern United States and eastern Mexico behave as different species when crossed, yet there is no break in its geographic range (Moore, 1946).

The case of *Crinia signifera* and *Crinia tasmaniensis* can be explained as a double invasion. The close similarity of the two species suggests a common ancestor in the not too distant past. One can imagine that the form ancestral to the present day *Crinia signifera* and *Crinia tasmaniensis* spread from the mainland to Tasmania. It is known that Tasmania was connected with the mainland at various times in the past, and as recently as the Pleistocene. Even with no land connection, Bass Strait should not be an insuperable barrier for a *Crinia*. Following this colonization, the two populations became isolated with the re-entry of ocean water in the Bass Strait area. With the passage of time, it can be imagined that the mainland and Tasmanian populations diverged and eventually became specifically different. A second invasion from the mainland, perhaps during the last land connection of the late Pleistocene, would bring the form we now call *Crinia signifera* in contact with the locally evolved island form we now call *Crinia tasmaniensis*.

Whether or not this is the correct explanation will never be known. All that can be said is that the hypothesis was made to fit the facts and a geological basis for the interpretation is available.

ACKNOWLEDGEMENTS

It is with sincere thanks that I remember my associates on the field trips that provided the material. These were Professor P. D. F. Murray, Mr. Allen Keast, Mr. and Mrs. Albert Main, Mrs. Eileen Finch, Miss Sally Moore, and Dr. Betty C. Moore. Mr. Donald McMichael collected the *Crinia signifera* from Mt. Kosciusko. The interest of Professor H. Waring made possible my trip to West Australia.

SUMMARY

The continent of Australia has a moist section along the east coast and another in the south-west corner. These two areas are separated by a broad arid zone that acts as an effective barrier for those species of amphibia that require a moist environment. *Hyla aurea* is found in both of the moist zones but is absent from the arid center. The two populations, which are now discontinuous, probably have not been connected in recent times. There has been almost no morphological differentiation during the period of isolation but genetic divergence has reached the point where the two populations are behaving as different species. Crosses of east Australia × south-west Australia individuals result in complete hybrid inviability.

Similar experiments have been conducted with *Crinia signifera*, a species showing the same type of distribution, and the results are nearly the same. Crosses of east Australia × south-west Australia individuals result in considerable inviability of the progeny.

On the island of Tasmania *Crinia signifera* and the very similar *Crinia tasmaniensis* occur. Crosses of east Australian *signifera* × Tasmania *signifera* gives normal progeny. Crosses of east Australia *signifera* × Tasmania

tasmaniensis results in complete hybrid inviability. These data suggest a double invasion of Tasmania by *signifera*. The first group evolved into *tasmaniensis* but the second has not diverged from the mainland type.

The south-west Australian analogue of the eastern *Hyla aurea* should be known as *Hyla raniformis* (Parker). No name is available for the southwest Australian species formerly included in *Crinia signifera*. A new name, *Crinia insignifera*, is proposed.

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Vol. LXXXVIII, No. 839 The American Naturalist March-April, 1954

COMBINING ABILITY OF CERTAIN CHROMOSOMES IN DROSOPHILA WILLISTONI AND INVALIDATION OF THE "WILD-TYPE" CONCEPT

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INTRODUCTION

The genotype of an individual is determined by the combination of genes in the chromosomes which this individual has received from its parents. Certain combinations of genes yield phenotypes which possess high fitness while other combinations produce adaptively less valuable phenotypes. The rules which govern the fitness of gene combinations are very little known. The present article reports the results of experiments designed to explore some of the problems in this field. One of these problems is the effect on fitness of heterozygosis for recessive lethals which commonly occur in natural populations of many species of sexual deploid organisms. Another problem concerns the interaction of the gene complex borne in a given chromosome with those in other chromosomes in the gene pool of the same population. Are there chromosomes which yield adaptively superior combinations with many or with all other chromosomes? Or, may a chromosome which produces relatively high fitness in combination with one chromosome be deleterious in combinations with other chromosomes? *Drosophila willistoni*, a species which is very common in the tropical zone of the Western Hemisphere, furnishes abundant materials for studies in this field.

EFFECTS OF RECESSIVE LETHALS IN HETEROZYGOTES

Natural populations of *Drosophila willistoni*, and of all other species of Drosophila so far studied in this respect, carry great stores of concealed, or potential, variability. Although most flies found in nature are themselves phenotypically rather uniform, their chromosomes are often lethal or semi-lethal when present in duplicate; many other chromosomes make the homozygotes sterile, or otherwise physiologically or morphologically abnormal. A large proportion of the chromosomes in the state of nature contain, then, recessive mutant genes or gene complexes which are concealed in heterozygous condition in normal flies, but which are deleterious in double dose (Pavan et al., 1951, Dobzhansky, 1951).

Whatever may be the effects in homozygous condition of the gene complexes which occur in the chromosomes of natural populations, they do not remain completely inactive in the heterozygotes. This has been first demonstrated for the "recessive" lethals. Dobzhansky and Wright (1941) and Wright, Dobzhansky, and Hovanitz (1942) showed that chromosomes which

are lethal in double dose are much less frequent in natural populations of *Drosophila pseudoobscura* than they would have been if the lethals were completely recessive. They inferred that many of the lethals are slightly deleterious in heterozygotes. An alternative possibility was that there may be some local inbreeding in natural habitats, or that both the incomplete recessivity and the local inbreeding may be operative. Stern et al. (1952) found that many of the sex-linked lethals, induced by X-ray or arising spontaneously in *D. melanogaster* are perceptibly deleterious in heterozygotes (average viability of a heterozygote was estimated to be approximately 96 per cent of the viability of zygotes carrying no sex-linked lethals). However, some of the lethals seemed to produce no detectable effects in heterozygotes, or produced ostensible improvements of the viability. Masing (1938) and Teissier (1942) have also claimed finding heterotic lethals in the same species.

Cordeiro (1952) tested the viability of heterozygotes for 52 different second chromosomes of *D. willistoni* which were known to be lethal when homozygous. These chromosomes were isolated from natural populations from several localities in Brazil. On the average, a lethal heterozygote had about 93 per cent of the viability of lethal-free zygotes. Prout (1952) obtained a similar result by comparing the frequency of lethal chromosomes found in the natural populations by Pavan et al. (1951) with the mutation rates producing lethals as determined in laboratory experiments by Dobzhansky, Spassky, and Spassky (1953). He estimated the average viability of heterozygotes for lethals in nature to be about 98 per cent of that of lethal-free zygotes. In view of the manifold sources of error, the agreement obtained by these completely independent methods is satisfactory, especially since the fitness need not necessarily be the same in experimental and in natural environments.

Statistical analysis of the data of Cordeiro (1952) has, however, disclosed the existence of a significant heterogeneity between the viability of heterozygotes for different lethals. Whether this meant that all lethals are deleterious although to different extent, or that some lethals are deleterious while others are neutral or even heterotic in heterozygotes could not be determined with certainty. Experiments were, therefore, arranged to test this point further.

ARE THERE HETEROTIC LETHALS?

Among the 52 second chromosomes which were lethal when homozygous and which were used in the experiment of Cordeiro (see above), 5 chromosomes were selected which seemed to give the highest viabilities in the heterozygotes. Of these, 3 chromosomes came from flies collected in the locality Quarai, in the state of Rio Grande do Sul, in southern Brazil. These chromosomes are denoted QL-6, QL-58 and QL-60. Two other lethals were derived from the population of Emboaba, in the same state; they are denoted EL-89 and EL-207. All the chromosomes have been perpetuated in balanced strains, in which the second chromosomes in question are

carried opposite a chromosome containing the second-chromosome dominant mutant gene Star, the recessives brown and abbreviated, and an inversion to suppress the crossing over (Pavan et al., 1951). Since the Star-brown-abbreviated chromosome is also lethal in double dose, the strains breed true, all the flies being phenotypically Star.

Apart from the chromosomes which are lethal to homozygotes, 8 chromosomes from the Quarai locality and 15 chromosomes from Emboaba which produce viable homozygotes are also perpetuated in strains balanced with the Star-brown-abbreviated marker. The chromosomes viable in double dose are designated Q-1, Q-5, etc, to E-633 (tables 1-4).

The experiments were arranged as follows. Males heterozygous for the Star-brown-abbreviated chromosome and for one of the chromosomes which are lethal when homozygous, were outcrossed to females homozygous for one of the non-lethal ("normal") second chromosomes. Usually about a dozen females and males were placed together, and then transferred daily to fresh culture bottles in which the females were allowed to oviposite for roughly 24 hours. This was repeated ten or more times, to obtain ten or more bottles with eggs deposited by the same group of parents. The progeny in these bottles was allowed to develop at the temperature of 25° C. When the adults hatched, counts were made in all bottles four times at 3-day intervals, the numbers of Star and wild-type (non-Star) flies being recorded. As a result, we had ten or more replicate cultures for each cross made; all cultures in which too few (fewer than 90–100) or too many (more than 300–320) flies hatched, were ignored, leaving exactly ten replications for each experiment.

The offspring of the cross Star/lethal × Normal/Normal fall into two classes: Star/Normal and lethal/Normal. The former class is phenotypically Star and the latter is wild-type. Theoretically, the two classes should appear in a ratio 1:1. However, the Star chromosome with its mutant markers decreases the viability of its carriers (see Pavan et al., 1951); the heterozygosis for a lethal may also modify the viability in various ways. In reality, the proportion of the wild-type class is in most instances slightly above 50 per cent (table 1).

Control experiments have been arranged by outcrossing Star/Normal males to Normal/Normal females, the experimental procedure being kept exactly like in the Star/lethal × Normal/Normal crosses. The offspring of the control crosses consist, of course, of Star/Normal and Normal/Normal flies, which would appear in a 1:1 ratio, if it were not for the mild deleterious effects of the Star chromosome. The results are summarized in table 2.

The difference between the experimental and the control progenies consists in that the wild-type flies in the former are heterozygous for a second chromosome which is known to be lethal when homozygous, while in the latter the wild-type flies are free of lethals. If heterozygosis for the lethals is deleterious, the proportion of the wild-type flies should be lower in the experimental than in the control cultures. If the lethals are beneficial

TABLE 1

CROSSES INVOLVING LETHAL AND QUASI NORMAL CHROMOSOMES
(Star/Lethal ♂ × Normal/Normal ♀)

Chromosomes	Flies Counted	Per Cent Wild Type	Heterogeneity Chi-Square
QL-6 × Q1	2504	52.36	4.79
QL-6 × Q5	1732	52.14	13.36
QL-6 × Q7	1571	56.84	21.48
QL-6 × Q11	2023	51.11	5.93
QL-6 × Q28	1526	51.44	12.30
QL-6 × Q31	1948	52.72	5.93
QL-6 × Q59	1744	53.04	10.55
QL-6 × Q1	2225	52.45	2.38
QL-6 × Q5	2344	53.07	6.74
QL-6 × Q7	2347	56.92	2.80
QL-6 × Q11	2323	50.84	12.69
QL-6 × Q28	2148	51.54	5.29
QL-6 × Q31	2366	54.48	4.93
QL-6 × Q59	2339	54.69	3.34
QL-58 × Q1	2224	57.33	4.62
QL-58 × Q5	1996	43.89	13.71
QL-58 × Q7	2411	54.42	6.56
QL-58 × Q11	2164	55.17	15.58
QL-58 × Q28	2809	52.22	6.49
QL-58 × Q31	2322	57.07	6.48
QL-58 × Q59	2280	53.90	11.29
QL-60 × Q1	2524	55.86	3.25
QL-60 × Q5	2586	54.60	8.92
QL-60 × Q7	2140	58.38	5.11
QL-60 × Q11	2274	54.75	4.70
QL-60 × Q28	2468	58.87	5.22
QL-60 × Q31	2594	59.60	16.55
QL-60 × Q59	2280	54.34	9.28
EL-89 × E59	2366	54.95	7.12
EL-89 × E216	1980	51.71	2.34
EL-89 × E401	2140	54.07	3.87
EL-89 × E429	2274	50.00	2.42
EL-89 × E443	2324	53.53	4.07
EL-89 × E472	2423	53.20	15.51
EL-89 × E482	2301	53.19	12.17
EL-89 × E510	2489	47.48	9.04
EL-89 × E538	2170	50.46	10.02
EL-89 × E583	2650	51.72	8.02
EL-89 × E617	2236	48.30	3.81
EL-89 × E616	2013	52.86	3.96
EL-89 × E619	2326	53.65	20.40
EL-89 × E633	2184	51.60	1.78
EL-207 × E59	2759	51.90	3.82
EL-207 × E216	2436	59.52	21.98
EL-207 × E401	2008	49.60	10.47
EL-207 × E429	2663	53.66	13.93
EL-207 × E443	2458	56.14	9.12
EL-207 × E472	2047	49.43	2.75
EL-207 × E482	2356	53.52	2.66
EL-207 × E510	2020	50.89	12.79
EL-207 × E538	2496	56.77	7.10
EL-207 × E583	2082	53.69	10.94
EL-207 × E616	2574	57.96	5.98
EL-207 × E617	2221	53.35	4.66

TABLE 1 (*continued*)

Chromosomes	Flies Counted	Per Cent Wild Type	Heterogeneity Chi-Square
EL-207 × E619	2451	56.71	5.58
EL-207 × E633	2628	55.13	4.57
EL-207 × E59	3402	51.54	11.06
EL-207 × E401	1879	52.21	9.33
EL-207 × E429	2085	51.08	9.11
EL-207 × E443	1943	51.11	11.46
EL-207 × E472	1880	50.11	8.33
EL-207 × E482	1747	55.81	5.29
EL-207 × E510	1893	53.09	5.76
EL-207 × E538	2352	52.78	8.55
EL-207 × E583	1863	51.64	12.27
EL-207 × E616	2211	58.79	14.52
EL-207 × E617	1989	53.49	13.14
EL-207 × E619	2557	56.00	4.09
EL-207 × E633	1629	54.69	4.52
TOTAL	154787	53.50	568.58

when heterozygous, the experimental cultures will show greater proportions of wild-type than the controls. A total of 154,787 flies were counted in the experiments involving lethals, and 53.50 per cent of them were wild-type. Among 26,669 flies counted in the control cultures, 52.38 per cent were wild-type. The lethals involved seem to be slightly beneficial when heterozygous; it should, of course, be recalled that they were picked out of a collection of lethals because they were suspected of being heterotic in heterozygous state.

A more detailed examination of the data is, however, necessary before the above conclusion can be accepted at face value. As stated above, 5 different lethals and 23 "normal" chromosomes are involved in the crosses, and the results need not be uniform for all these chromosomes; the chromosomes came from two distinct localities (Quarai and Emboaba); some of the experiments were carried out in the genetics laboratory of Columbia University and others at the University of Texas; finally, since every cross was replicated ten times, one can investigate the possible influence of the environmental conditions in different cultures.

Tables 1, 2, and 4 show the chi-squares for heterogeneity between the replicate cultures of each cross. Each of these chi-squares has 9 degrees of freedom. For 9 degrees of freedom, a chi-square of 4.168 corresponds to a probability of 0.9, 8.343 to 0.5, 16.919 to 0.05, and 21.666 to 0.01. It is clear, then, that no significant heterogeneity is observed between different cultures in most crosses. In fact, some of the chi-squares for replications are surprisingly small; however, summing them up, one obtains a combined chi-square for heterogeneity of 1114.50, which has 1098 degrees of freedom. This corresponds to a probability of about 0.36. The absence of significant heterogeneity between replicate cultures in most experiments is not unexpected. The Star as well as the wild-type classes in these experiments are heterozygotes, since they carry two different

TABLE 2

CROSSES INVOLVING QUASI-NORMAL CHROMOSOMES
(Star/Normal ♂ × Normal/Normal ♀)

Chromosomes	Flies Counted	Per Cent Wild Type	Heterogeneity Chi-Square
Q28 × Q1	1458	52.61	5.07
Q41 × Q28	2210	50.86	2.90
Q11 × Q31	1914	54.18	8.57
Q41 × Q59	1952	55.69	8.14
Q59 × Q11	2108	51.76	7.49
Q28 × Q5	2786	48.56	6.61
Q31 × Q7	1618	53.28	9.21
E429 × E583	2143	50.96	5.76
E619 × E429	2240	51.83	8.98
E583 × E443	2270	52.91	7.17
E510 × E59	2075	53.20	9.74
E59 × E401	2109	53.72	4.42
E401 × E617	1786	51.34	1.34
	26,669	52.38	85.40

second chromosomes. As shown by Dobzhansky and Wallace (1953), heterozygotes have more complete homeostatic adjustments than do homozygotes, and consequently show uniform survival rates despite the environmental variations in different cultures.

In Table 2, the crosses involving Quarai chromosomes (Q) are listed first, and those involving Emboaba chromosomes (E) second. The former have yielded 52.12 ± 0.42 and the latter 52.32 ± 0.45 per cent of wild-type flies. The difference is not significant. In Tables 2 and 4 the series of crosses performed at Columbia University is listed first, and the repetitions carried out at the University of Texas are shown second. The results of similar crosses made in the two laboratories are alike within limits of sampling errors; the t values for the Quarai and the Emboaba lethals correspond to probabilities of 0.8 and 0.9 respectively.

The viability is, however, by no means uniform when the same chromosome which is known to be lethal when homozygous is combined in heterozygous condition with different non-lethal chromosomes. Thus, lethal QL-6 has been tested with seven different normal chromosomes, the different tests yielding from 50.8 to 56.9 per cent wild-type (table 1). As shown in table 3, this heterogeneity is highly significant; the chi-square of 34.97 corresponds, for 6 degrees of freedom to a probability much less than 0.001. The four other lethal chromosomes tested behave like QL-6, in that they produce heterogeneous results in combinations with different non-lethal chromosomes (table 3, first five lines above). Thus, EL-207 gave from 49.4 to 59.5 per cent wild-type in different combinations (table 1).

Whether a chromosome which is lethal when homozygous is or is not heterotic when heterozygous may, accordingly, depend upon the properties of the chromosomes with which it is combined. Table 3 shows that the lethals QL-60 and EL-207 produced respectively 55.8 and 54.7 percent

TABLE 3

UNIFORMITY OR HETEROGENEITY PRODUCED WHEN CERTAIN CHROMOSOMES ARE PRESENT IN HETEROZYGOTES WITH OTHER CHROMOSOMES FROM THE SAME POPULATION

Chromosome	Number of Combinations Tested	Total Flies	Per Cent Wild	Heterogeneity Chi-Square	Probability
QL-6	7	29,162	53.08	34.97	< 0.001
QL-58	7	16,201	53.60	107.28	< 0.001
QL-60	7	16,919	55.83	22.24	0.001
EL-89	14	31,886	51.85	55.29	< 0.001
EL-207	13	58,193	54.68	244.24	< 0.001
Q-1	3	5,871	38.65	13.04	0.001
Q-5	3	5,728	41.18	22.60	< 0.001
Q-7	3	5,180	38.65	16.05	< 0.001
Q-11	3	5,396	42.01	10.60	0.06
Q-28	8	14,304	39.43	74.62	< 0.001
Q-31	3	5,629	39.60	0.86	0.7 – 0.6
Q-41	2	3,934	44.26	0.70	0.7
Q-59	3	6,046	40.14	27.67	< 0.001
E-59	3	6,341	37.25	9.75	< 0.01
E-216	3	6,506	36.72	2.38	0.3 – 0.2
E-401	3	5,915	38.50	2.13	0.3 – 0.2
E-421	3	6,920	39.34	19.54	< 0.001
E-429	2	3,942	38.41	3.13	0.08
E-443	2	3,881	38.06	1.26	0.25
E-472	3	5,986	39.17	10.68	< 0.01
E-482	3	5,617	37.35	10.63	< 0.01
E-510	3	5,801	36.25	2.07	0.4 – 0.3
E-538	3	5,739	38.18	27.35	< 0.001
E-583	3	6,437	37.35	16.30	< 0.001
E-616	2	4,260	37.56	1.25	0.25
E-617	3	5,408	37.06	3.54	0.2 – 0.1
E-619	3	7,155	39.37	19.26	< 0.001
E-633	13	28,367	36.96	34.08	< 0.001

wild-type among 16,919 and 58,193 flies counted. The control crosses, not involving lethal chromosomes, yielded 52.4 percent wild-type among 26,669 flies, which is a significantly lower percentage than observed when the lethals were present. The average effects of QL-60 and EL-207 in combination with the chromosomes with which they were tested in our experiments are, thus, heterotic. But, as stated in the foregoing paragraph, some of the crosses involving QL-60 and EL-207 have given only 50.8 or 49.4 per cent wild-type. In these crosses, QL-60 and EL-207 are, then, deleterious to the heterozygotes. On the other hand, EL-89 gave, in fourteen different combinations with other chromosomes, an average of 51.85 per cent wild-type, which is slightly below the control value (cf. tables 3 and 2). The lethal EL-89 is, then, neither heterotic nor deleterious in heterozygotes. Table 1 shows, however, that the crosses involving EL-89 gave from 47.5 to 54.95 per cent wild-type; accordingly, EL-89 is somewhat deleterious in some and heterotic in other combinations.

Cordeiro (1952) and Prout (1952) found that the recessive lethals in second chromosomes of natural populations of *Drosophila willistoni* are,

on the average, deleterious in heterozygotes. The fact that four out of the five lethals tested in the experiments described in the present article tend to be heterotic more often that deleterious does not contradict the findings of Cordeiro and Prout. As stated above, these five lethals are not a random sample of lethals found in natural populations; they were chosen because they were suspected of being heterotic, and the suspicion was confirmed for four of them.

TABLE 4

CROSSES INVOLVING QUASI-NORMAL CHROMOSOMES

(Star/Normal ♀ × Star/Normal ♂)

Chromosomes	Flies Counted	Per Cent Wild Type	Heterogeneity Chi-Square
Q28 × Q1	2025	35.5	9.60
Q28 × Q5	1842	37.6	11.96
Q28 × Q7	1917	35.6	15.51
Q28 × Q11	1711	43.3	4.46
Q28 × Q31	1345	40.3	3.77
Q28 × Q59	1837	36.8	21.32
Q28 × Q5	1986	45.1	7.22
Q28 × Q7	1641	42.2	11.10
Q7 × Q11	1622	38.7	7.04
Q11 × Q41	2063	43.6	5.17
Q41 × Q59	1871	44.9	1.97
Q59 × Q31	2338	38.9	12.81
Q31 × Q1	1946	40.0	7.43
Q1 × Q5	1900	40.6	10.70
E633 × E619	1909	38.1	5.59
E633 × E59	1744	37.4	4.91
E633 × E216	1830	35.3	6.02
E633 × E401	2184	37.8	10.55
E633 × E421	2338	37.4	23.54
E633 × E443	1976	37.2	8.26
E633 × E472	1653	37.5	6.62
E633 × E482	2002	34.5	4.79
E633 × E510	1373	37.2	4.83
E633 × E538	1772	33.8	3.82
E633 × E617	1229	35.2	5.52
E633 × E583	2129	34.0	5.56
E633 × E616	2339	38.3	7.17
E216 × E472	2036	37.6	10.93
E472 × E538	2297	41.8	19.95
E538 × E401	1670	37.8	1.83
E401 × E429	2061	39.7	28.16
E429 × E510	1881	37.0	27.48
E510 × E59	2547	35.2	6.34
E59 × E583	2050	39.7	20.92
E583 × E617	2258	35.4	28.11
E617 × E616	1921	36.7	11.00
E616 × E633	2179	40.7	12.02
E633 × E482	1710	38.9	10.28
E482 × E443	1905	38.9	12.56
E443 × E619	2606	42.7	20.86
E619 × E216	2640	37.0	33.90
	80,283	38.51	471.58

COMBINING ABILITY OF NON-LETHAL CHROMOSOMES

Table 4 summarizes the results of crosses in which 23 different chromosomes which were known to produce normally viable homozygotes were tested in 41 different combinations. The crosses were between females and males which carried the Star marking chromosome and different "normal" chromosomes (Star/Normal A ♀ × Star/Normal B ♂). Non-Star individuals which are obtained in the offspring of such crosses are, of course, heterozygotes from two different "normal" chromosomes (Normal A/ Normal B).

The data may be analyzed best by comparing the outcomes of crosses in which a certain chromosome is a participant. The number of crosses studied varied from 2 (for Q-41, E-429, E-443, and E-616) to 13 (for E-633, table 4). Chi-squares for heterogeneity have been computed for the combinations in which each of the 23 chromosomes was a participant. These chi-squares are listed in Table 3. The number of the degrees of freedom is in each case one less than the number of combinations tested. It can be seen that 13 out of the 23 chromosomes gave heterogeneous results, although some of them were tested in only two or three combinations. The fitness of a "normal" fly depends, then, upon which combination of chromosomes this fly carries.

It may be noted (table 3) that one of the chromosomes, Q-41, gives a fairly high average viability (44.3 per cent wild-type), while another chromosome, E-633, gives a low average viability (37.0 per cent wild-type). It does not follow, however, that Q-41 is heterotic and E-633 is deleterious. Indeed, the same chromosome may be favorable in some combinations and unfavorable in others. No chromosome has been tested in a sufficient number of combinations to enable us to judge its average, or "general," combining ability.

DISCUSSION

Except in polymorphic species, individuals which occur in natural populations of most organisms are sufficiently uniform in phenotype to be described simply as "normal," or representing the "wild-type" of their respective species or races. It is legitimate to use the concept of "norm" to facilitate the description of mutants and of genetical and environmentally induced aberrations that occur from time to time. Unfortunately, some biologists have gone beyond this, and came to regard the "norm" as a sort of ideal prototype of which the actually existing individuals are imperfect copies. This typological thinking, the roots of which go down to the Platonic philosophy, is basically anti-evolutionistic, and has produced much confusion in biological thought.

In 1927, Chetverikov discovered that many individuals in nature are heterozygous carriers of recessive mutant genes, most of which are sharply deleterious, or even lethal, in double dose. This discovery, amply confirmed by subsequent investigators, did not at first appear to discredit the concept of the wild-type. The recessive mutants, regardless of what may be their effects when homozygous, are concealed in natural populations in hetero-

zygous state. Their carriers continue to be phenotypically normal. The finding that some of the concealed mutants may be incompletely recessive may still be harmonized with the idea of the wild-type. Indeed, if the concealed mutants were to be removed from the gene pool of a population, this population would come to consist of entirely normal (or supernormal?) individuals. The normality so conceived represents, however, a description of an imaginary ideal, rather than of any actually observed, population.

It became even more difficult to maintain the concept of normality when Pavan et al. (1951), Dobzhansky and Spassky (1953, 1954), Wallace et al. (1953) and Wallace and Madden (1953) found that it is not an exceptional chromosome which causes deviations from normality when homozygous, but that a decided majority of the chromosomes in natural populations of species of Drosophila produce more or less aberrant individuals when present in double dose. The "norm" is, then, a condition which is found in individuals in which the two chromosomes of each pair would cause a more or less serious physiological or morphological disturbance if permitted to become homozygous. In other words, the adaptive welfare of sexual and cross-fertilizing species is based on a wide prevalence in nature of heterozygosis for gene alleles or gene complexes which are deleterious when homozygous.

The basic fact which is revealed by the data presented in this article, and also in those of Wallace (1952) and Wallace and King (1952) on *Drosophila melanogaster,* is that the genetic variability which is strikingly apparent when the chromosomes of "normal" flies become homozygous is not entirely concealed in the heterozygotes. Tables 1–4 show conclusively that different combinations of chromosomes derived from natural populations produce a variety of genotypes with different norms of reaction in a standardized environment. "Normal" heterozygotes are, then, a complex of genotypically as well as phenotypically diversified individuals, which have in common only the absence of gross aberrations and teratological traits. It is clear without further comment that no one genotype and no one phenotype can legitimately be regarded as typifying the species or a population. A normal Drosophila, like a normal man, is an abstraction. This abstraction may be used to facilitate description of genetic and environmentally induced malformations, but beyond this it rapidly becomes misleading. An example of such misleading use is the setting up of standards of "normal" weight for all men, or for that matter for men of a given age and height. Different genotypes react to different environments by development of different body weights. This need not prevent one from trying to discover which body weight is optimal for a given genotype in man, or which temperature, nutrition, and degree of crowding is optimal for a given Drosophila genotype.

Little can be said at present concerning the interrelations between the effects produced by a given gene complex in homozygous condition on the one hand, and in heterozygotes with other gene complexes on the other. The term "combining ability," widely used in the literature on animal and

especially on plant breeding, is really a confession of ignorance of the phenomena involved. To say that a heterozygote has a high, or a low, viability because the chromosomes possess high, or low, combining ability is like saying that an animal or a plant grows because it has a growing ability. Only some few empirical rules are beginning to emerge. Thus, we know that a majority of the chromosomes which are lethal when homozygous are somewhat deleterious also in heterozygotes. However, some of the lethals found in natural populations are not deleterious or are even heterotic. The viability which a heterozygote for a lethal may have is a function of the gene complex in the chromosome which is the mate of the lethal-carrying one, and, no doubt, also of the rest of the genotype. For the time being we can not predict which chromosome will give the most viable heterozygote with a certain lethal-bearing chromosome. Combinations of different chromosomes which are not lethal when homozygous also produce a diversity of heterozygotes with different viabilities, and the fitness given by a chromosome A with the chromosome B does not allow prediction of the fitness which either of them might produce with C. Discovery of the genetic and physiological basis of "combining ability" requires further studies.

ACKNOWLEDGMENTS

The experiments described in this paper have been carried out while one of us (A. R. Cordeiro) held a research fellowship provided by the Rockefeller Foundation, and worked as a guest of the Department of Zoology of Columbia University and the Genetics Institute of the University of Texas. The generous help of Mrs. Malvina Cordeiro in the conduct of the experiments and of Mrs. O. A. Pavlovsky and T. D. Gidaspow in making the calculations is gratefully acknowledged.

SUMMARY

Five second chromosomes which were known to be lethal in double dose, and 23 chromosomes producing about normally viable homozygotes, were chosen from samples of chromosomes from two South-Brazilian populations of *Drosophila willistoni*. The lethal chromosomes were selected among a much larger collection of lethals because they seemed to produce exceptionally vigorous heterozygotes with other chromosomes from the same populations. The quasi-normal chromosomes were picked out at random.

Heterozygotes with various combinations of lethal and quasi-normal chromosomes were produced by appropriate crosses. The viability of these heterozygotes was measured by observing the ratios of individuals which carried the chromosomes to be tested and individuals carrying chromosomes with suitable marking mutant genes. Some heterozygotes for lethal and quasi-normal chromosomes proved more viable than heterozygotes which did not carry chromosomes known to be lethal when homozygous. A chromosome which is lethal in double dose may, therefore, be heterotic when present in a heterozygote. However, two chromosomes which give a highly viable heterozygote may produce either a high or a low viability in hetero-

zygotes with a third chromosome. This is true for chromosomes which are lethal when homozygous, as well as for chromosomes which give quasi-normal homozygotes. The combining ability of chromosomes may be measured empirically, but the rules which might permit prediction are unknown at present.

Natural populations of Drosophila, and presumably of other sexual species, contain great stores of chromosomes with different complexes of genes. An individual carries, then, two chromosomes of each pair chosen at random from the gene pool of the population. No one of the resulting variety of genotypes and phenotypes can be regarded as "normal," or representing the "wild-type" of the species or population. The "wild-type" is an abstraction the usefulness of which is limited to serving as a point of reference in describing genetic and environmentally induced aberrations.

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Vol. LXXXVIII, No. 839 The American Naturalist March-April, 1954

INTRASPECIFIC SPERM COMPETITION IN *LEBISTES RETICULATUS*

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It has long been known that females of the ovoviviparous Poeciliid fishes retain viable spermatozoa within the ovary for many months. As many as eight broods of young (at about 30 day intervals) from one female have resulted from exposure to a male for only a day or two. When a second male becomes involved, a question arises as to what proportion of the next offspring are due to the more recent fertilization. Winge (1937) concluded in Lebistes that the old spermatozoa cannot compete with the fresh ones and that ordinarily a mixed brood is only obtainable when both males are together with the female at the same time. Vallowe (1953) found in a series of matings of Xiphophorins that after a female has been fertilized by a male of either the same or a different species, sperm from a second male can also fertilize ova which are in the same broods. Much variation in the proportion of offspring due to either male was observed. However, Clark (1950) found that interspecific sperm competition appears to be a significant factor as a sexual isolating mechanism between *X. maculatus* and *X. belleri*. Mixed sperm give primarily offspring of the species of the parental female rather than hybrids when artificial insemination is employed.

Turner (1937) in an extensive study of reproductive cycles in Poeciliid fishes found the fertilization of a group of ova in Lebistes taking place 8 days subsequent to the birth of the previous brood. Further observation indicated that all ova of a group are fertilized within 2 days; embryos develop during the next 20 days and are voided. This constitutes the typical 30 day cycle. Dildine (1936) states that ova are fertilized from 9 to 12 days after the birth of a previous brood, and that about 20 more days are required for gestation. Haempel (1950) found an autogenous rhythm of 4 to 7 days in the estrous cycle of female Lebistes which would explain the lag in successive fertilizations observed above. It is apparent that any male copulating with a female within a week of the birth of a previous brood would be likely to father offspring in the next brood. Whether sperm from a second male Lebistes introduced to a female within a few days after bearing young would in all cases succeed over sperm already waiting in the ovary was a question which led to the present series of experimental matings.

MATERIALS AND METHODS

Three xanthic mutant phenotypes of Lebistes, cream, blond, and gold, each affecting overall body coloration and recessive to the wild-type, were

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used. Matings were made of mature virgin females paired to males of the same strain. Males were removed after about 20 days in each case. After birth of the first brood, offspring were removed and a male of a dominant phenotype homozygous for body color genes was introduced for about 20 days. Offspring of the second brood due to either male could readily be identified at birth. Male contact for a period of a week or less in each case would probably have been sufficient to effect fertilization. In this connection, Clark and Aronson (1951) found that inseminations are not effected during the commonly observed non-contact and momentary contact thrusts, but only when the female specifically halts in her swimming. This is probably why Vallowe (1953) failed to get broods of Xiphophorus by attempting to mate a female with two male fish in rapid succession. In the present experiment no attempt was made to mate virgin female Lebistes with two males in rapid succession, or to administer mixed sperm by artificial insemination. Pairing two males successively with a virgin female for 8 days each (completed estrous cycles) might give results different from those observed below in the second broods in a series of matings, since both groups of sperm would have been stored for some time when the next ova became mature following gestation. These aspects remain to be studied.

RESULTS

The results of a series of ten matings begun concurrently are shown in Table 1. As expected, all offspring of the first broods were of the parental phenotype. The second broods derived from the four cream females gave variable results. In two instances none of the offspring came from fertilizations by sperm of the second male. It is possible that both of these males were infertile, but more likely that their sperm were weaker or less numerous than those already stored in the ovary. In matings #1 and #4, however, the sperm from the second male dominated completely. The cream females died before additional broods were born. Lack of hardiness and reduced longevity are characteristic of this double recessive mutant. Matings with the four blond females provide much better data on sperm competition. In the second broods, sperm from the second male predominated in three cases and fertilized all ova in the fourth. In succeeding broods of matings #5, #7, and #8, the wild-type sperm completely excluded the sperm of the original blond male. However, in mating #6 the unexpected happened. Succeeding broods show that the sperm of the first blond male gradually excluded those of the second male, until only blond offspring were produced in the fifth brood. In the remaining two matings involving gold females, one female died early, while sperm from the second male completely succeeded over those of the first male in the final mating.

DISCUSSION AND SUMMARY

These matings indicate that sperm competition within this species does not always mean complete success for sperm from the more recent copulation,

TABLE 1

RESULTS OF SUCCESSIVE MATINGS INDICATING SPERM COMPETITION

Parental phenotypes Date male introduced			Phenotype and number of young Date of birth				
Female	Male 1	Male 2	1st brood	2nd brood	3rd brood	4th brood	5th brood
1. Cream	Cream 10/31/50	Wild-type 12/4/50	all cream 12/2/50	10 wild-type 0 cream 1/17/51	11 wild-type 0 cream 3/2/51	female died	....
2. Cream	Cream 10/31/50	Gold 12/5/50	all cream 12/5/50	0 gold 7 cream 1/26/51	female died	....	....
3. Cream	Cream 10/31/50	Wild-type 12/6/50	all cream 12/6/50	0 wild-type 4 cream 1/24/51	female died	....	....
4. Cream	Cream 10/31/50	Wild-type 12/22/50	all cream 12/22/50	15 wild-type 0 cream 1/29/51	female died	....	....
5. Blond	Blond 10/31/50	Wild-type 12/4/50	all blond 12/4/50	17 wild-type 1 blond 1/11/51	15 wild-type 0 blond 2/25/51	12 wild-type 0 blond 3/30/51	11 wild-type 0 blond 5/2/51
6. Blond	Blond 10/31/50	Wild-type 12/6/50	all blond 12/6/50	17 wild-type 7 blond 1/8/51	3 wild-type 8 blond 2/6/51	1 wild-type 13 blond 3/10/51	0 wild-type 13 blond 4/16/51
7. Blond	Blond 10/31/50	Wild-type 12/22/50	all blond 12/22/50	16 wild-type 2 blond 1/15/51	21 wild-type 0 blond 2/13/51	20 wild-type 0 blond 3/18/51	17 wild-type 0 blond 4/17/51
8. Blond	Blond 10/31/50	Wild-type 12/22/50	all blond 12/22/50	10 wild-type 0 blond 11/23/51	17 wild-type 0 blond 2/24/51	14 wild-type 0 blond 3/30/51	11 wild-type 0 blond 5/1/51

TABLE 1 *(continued)*

Parental phenotypes Date male introduced			Phenotype and number of young Date of birth				
Female	Male 1	Male 2	1st brood	2nd brood	3rd brood	4th brood	5th brood
9. Gold	Gold 10/31/50	Wild-type 12/5/50	all gold 12/5/50	female died	....	....	....
10. Gold	Gold 10/31/50	Wild-type 12/13/50	all gold 12/13/50	7 wild-type 0 gold 2/19/51	10 wild-type 0 gold 3/13/51	....	....

even though this is often the case. Rather it is probable that sperm competition depends upon the potency of the individual males. However, when sperm from two sources are of similar potency, the fresher ones will predominate in fertilizing the ova. The result in mating #6 might be attributed to a difference in the receptivity of the female during the periods when she was paired with the two males. If she was less receptive when the wild-type male courted her, he may have inseminated her only once and the concentration of his sperm could have therefore been much lower. This explanation however seems unlikely in view of the fact that the mating period in each instance extended over 20 days. It is conceivable also that mutant females may at times favor sperm from their own strain because of some specific interaction, but this has not been tested. No anatomical differences have been noted among these mutants.

A comparison of the mating dates and birth dates in Table 1 shows that there is considerable variation in the reproductive cycles of females even under similar living conditions. Although cream females #1 and #4 were placed with males at the same time, there was a difference of 20 days in the birth date of the first broods. The males are always eager to copulate. Variations in the reproductive cycle probably have little effect on sperm competition, however.

From a practical standpoint, it is apparent that geneticists and fish breeders working with Lebistes or other ovoviviparous fishes should use virgin females in all matings where pure stocks are important. Otherwise distinct color markings might be used to segregate offspring of non-virgins when the phenotype of the original male(s) is known with certainty.

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Vol. LXXXVIII, No. 839 The American Naturalist March–April, 1954

SEXUAL ISOLATION BETWEEN *DROSOPHILA PERSIMILIS* AND *DROSOPHILA PSEUDOOBSCURA*[1]

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The two closely related species, *Drosophila pseudoobscura* and *Drosophila persimilis,* have been considered to be good species, (Dobzhansky and Epling, 1944) primarily because of the sexual isolation existing between them rather than because of any morphological differences, although methods have been discovered (Mather and Dobzhansky, 1939; Reed, Williams, and Chadwick, 1942; Reed and Reed, 1948; Rizki, 1951) by which the two species can be distinguished morphologically. Because of this situation, there has been considerable study of the nature of the sexual isolation between these two species (see Dobzhansky, 1951a and Patterson and Stone, 1952 for discussion and references). The studies thus far have shown that, while these species are sexually isolated to some extent under experimental conditions, that nevertheless hybridization does occur in the laboratory. However, no evidence has been found as yet for hybridization in nature. These facts would seem to point to factors other than the mating process itself as being responsible for the complete sexual isolation observed in nature. However, the experiments on sexual isolation between these two species have depended for the most part on what has been termed (Merrell, 1950) the "male multiple choice" technique with the males of one species being given an opportunity to mate with females of both species. The spermathecae of these females are then examined to determine what proportion of each species has been inseminated, and hence to determine the degree of sexual isolation. This procedure suffers from the handicap that the behavior of the female can not be adequately studied. While the male has the opportunity to mate with females of his own species or of the other species, the female has only the "choice" between mating or not mating.

The indications are, however, that within a species or among closely related species, the female determines whether or not copulation is to occur. Streisinger (1948) in a short but significant paper showed that males of pseudoobscura, given a choice of mating with etherized females of their own species or of persimilis, mated at random, inseminating about half of the females of each species. However, of the unetherized females, 90% of pseudoobscura and only 10% of persimilis were inseminated. A similar experiment, involving less closely related species, namely melanogaster males and melanogaster and persimilis females, again resulted in random mating with the etherized females but complete sexual isolation when the

[1]These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and University of Minnesota, NR 164-111.

females were not etherized. Merrell (1949a) has shown that in *D. melanogaster* mating was more nearly at random with male than with female "choice" when mutants and wild type flies were used, and also (1949b) that with different wild type strains of *D. melanogaster*, the length of the period of courtship was characteristic of the females but the duration of copulation was characteristic of the males. These results indicate the primary role of the female in determining whether or not copulation is to occur.

In view of this conclusion, it seemed desirable to develop some method for testing "female multiple choice" in *D. persimilis* and *D. pseudoobscura*. The examination of the spermathecae is, of course, not feasible unless a method can be devised for distinguishing between the sperm of these two species. It is possible that the insemination reaction is different in the inter- and intra-specific matings, but this point has not been investigated, and it might well be difficult to score for such closely related species.

MATERIALS AND METHODS

The method used involved mating between mutant stocks of the two species, orange of *D. persimilis*, and glass of *D. pseudoobscura*, which were very kindly furnished by Dr. Boris Spassky of Columbia University. With these mutant stocks, it became possible to test male multiple "choice," female multiple "choice," and multiple "choice" with males and females of both species present.

A major drawback to the use of mutant stocks is the possibility that the mutant genes may so reduce viability or modify mating behavior that the true picture of the situation with regard to sexual isolation may be obscured. Mutants in other species have been shown to cause selective mating within the species (Merrell, 1949a). While this result is clearly different from sexual isolation, it may complicate experiments in which sexual isolation is also involved (Merrell, 1950). Therefore, it seemed desirable to get some idea of the relative viability of these mutants as compared to their own wild types.

The flies were raised on the Cream of Wheat medium described by Dr. Spassky in D.I.S. 17, modified by the addition of 100 grams of dried brewer's yeast. A drop or two of live yeast suspension was also added at the time the flies were placed in the bottles. All of the flies used in these experiments were raised in a constantly lighted room at 21 ± 1°C with 50% relative humidity.

The results of the viability tests are shown in table 1. It can be seen that under uncrowded conditions in half pint culture bottles, the *or* mutants of *D. persimilis* appeared in the expected numbers from a cross of heterozygous males and females although there were significantly fewer males than females of both types. However, the homozygous *gl/gl* flies were significantly less frequent than expected from a similar cross, and in *D. pseudoobscura* again there were fewer males than females. Hence, *gl* can be said to be relatively less viable than *or* when each is compared to its

TABLE 1

VIABILITY OF MUTANT STOCKS OF *D. PSEUDOOBSCURA* AND *D. PERSIMILIS* COMPARED TO THEIR WILD TYPES

Genotype	Males	Females	Total	χ^2
	D. pseudoobscura (From +/gl × +/gl)			
+/+ and +/gl	415	496	911 (80.2%)	
gl/gl	92	133	225 (19.8%)	16.34**
	D. persimilis (From +/or × +/or)			
+/+ and +/or	478	657	1135 (73.9%)	
or/or	171	230	401 (26.1%)	1.00

own wild type under these culture conditions. However, *D. pseudoobscura* of both wild and mutant type seemed to do somewhat better under these conditions than *D. persimilis*. In any case, the viability differences did not seem great enough to cause any major difficulties in the mating tests.

The flies used in the mating experiments were reared in half pint culture bottles, and shortly after emergence, the virgin males and females were separated and placed in creamers. The flies were then aged in isolation from all but members of their own species and sex for periods of at least 6 days but not more than 11 days before the matings were made. For the mating tests, usually 10 flies of each type were placed together in a creamer. Occasionally less than 10 of each type were available so that as few as 6 of each type were used, but in all cases equal numbers of each kind were present at the outset. The males and females remained together for two days after which each female was placed in a separate creamer, and the males were discarded. The type of mating was determined from the progeny of the female. The possibility that the interspecific matings would not be detected by this method was not great because the hybrid wild type progeny were usually more numerous and viable than the mutant progeny from the intraspecific matings.

RESULTS

The results with the male multiple "choice" experiments are shown in table 2. Males of both persimilis and pseudoobscura mated more often with

TABLE 2

MALE "CHOICE"

Males	Females	Homogamic		Heterogamic		Isolation Estimate
		n	%	*n*	%	
Persimilis (or)	persimilis (or) pseudoobscura (gl)	45	73.3	46	39.1	.457
pseudoobscura (gl)	persimilis (or) pseudoobscura (gl)	41	90.2	42	33.3	

females of their own species than with females of the other species. In each case, many more than half of the females of the same species were inseminated, but only about a third of the females of the other species were inseminated. Thus, though sexual isolation was clearly in evidence, there was a fairly high frequency of heterogamic mating in the male "choice" experiments. The isolation estimate, I = 0.457 (with 1.0 indicating random mating and 0.0 complete isolation, Merrell, 1950) indicates the degree of sexual isolation, and there is a statistically highly significant deviation from random mating due to sexual isolation ($\chi^2 = 14.2$; $p < 0.01$).

When females of one species were placed with males of both species, sexual isolation was again in evidence (table 3), but was much more complete than with male "choice" (I = 0.069; $\chi^2 = 58.3$). When males and females of both species were present in equal numbers, the results (table 4) were essentially the same as those with female "choice" (I = 0.058; $\chi^2 = 58.6$).

Thus, on the basis of these experiments, it must be concluded that the ability of these females to discriminate between males of their own species and males of a different species when given the opportunity to mate with

TABLE 3

FEMALE "CHOICE"

Males	Females	Homogamic		Heterogamic	Isolation estimate
		n	%	%	
persimilis (or) pseudoobscura (gl)	persimilis (or)	46	65.2	4.3	.069
persimilis (or) pseudoobscura (gl)	pseudoobscura (gl)	50	84.0	6.0	

either type, is considerably greater than that of the males when given a similar "choice" between females. In fact, it might even be argued, on the basis of Streisinger's results, that the major reason for the deviation from random mating in the male "choice" experiments may also be the behavior of the females. It suffices to say at present, however, that the degree of sexual isolation between *D. persimilis* and *D. pseudoobscura* is considerably greater in female "choice" experiments than in male "choice" experiments, and that the degree of sexual isolation when males and females of both species are together is similar to that in the female "choice" experiments. Since, when both sexes had the opportunity to mate with members of their own species in the multiple "choice" tests, sexual isolation was no more pronounced than in the female "choice" tests, the sexual isolation observed in the various tests must be due primarily to the behavior of the female.

It should be noted that the differences measured appear to be due almost entirely to sexual isolation rather than to selective mating since the male and female mating ratios (Merrell, 1950) based on tables 2, 3, and 4 were

TABLE 4

MULTIPLE "CHOICE"

Males	Females	Homogamic		Heterogamic	Isolation estimate
		n	%	%	
persimilis (or)	persimilis (or)	49	71.4	0.0	.058
pseudoobscura (gl)	pseudoobscura (gl)	51	66.7	7.8	

not significantly different from 1. However, as might be expected on the basis of previous work by others, the persimilis females mated somewhat less frequently than the pseudoobscura females (114/138).

DISCUSSION

A variety of terms has been used to categorize the two major types of mating behavior which have been observed. To clarify to some extent the usage of these terms, it should be noted that (positive) "assortative mating" (Pearson and Lee, 1903) or "courtship discrimination" in the sense of Spieth (1951) lead toward sexual isolation, while "preferential mating" (Pearson and Lee, 1903), differences in "sexual drive" (Spieth, 1951), "differential mating propensities" (Bateman, 1949), "one-sided mating preferences" (Dobzhansky and Streisinger, 1944), or "selective mating" (Rendell, 1945; Merrell, 1949) produce changes in the relative frequencies of the genes involved, and hence must be considered as a phase of natural selection. For this reason, selective mating would seem to be the most useful and descriptive term to designate the second type of mating behavior.

The results of the experiments would seem to support the conclusion drawn by Merrell (1949a) and by Bateman (1949) as to the primary role of the female in determining whether or not mating is to occur, and hence in determining the degree of sexual isolation between closely related species. This conclusion was correctly interpreted by Spieth (1951), but was later misquoted (Spieth, 1952) as a belief that species discrimination is solely a function of the male. On the basis of his work, Spieth (1952) has stated (p. 464) that "species discrimination is a function of both sexes," among closely related species. In support of the importance of the male, he cited the behavior of *D. virilis* males in breaking off courtship with females of other species of the virilis group. In this situation, the male does play a role in sexual isolation, but there can be little knowledge of whether or not mating would have occurred with any degree of frequency even if the male had persisted in courtship. Spieth also wrote, (p. 462) "Despite the fact that she (the female) will copulate more than once during her lifetime, the ratio of acceptances to rejections of the males' courting attentions must be extremely low." Further on (p. 469), he states, "....in the courting stage at least, the males appear to have a low threshold of response, while the females may display a high threshold of response. This results in the

conservation of male gametes, since females whose threshold of response is so high that they are non-receptive to the males' overtures are able in all instances to prevent the male from achieving copula." From these last two statements, it is clear that Spieth's views are essentially similar to those of the author concerning the primary role of the female. For if, no matter what the behavior of the male may be, the female determines whether or not mating is to occur, then her role must be the more significant one. This conclusion has been reached with regard to the sexual isolation between *D. persimilis* and *D. pseudoobscura* by Dobzhansky (1951) on the basis of indirect evidence, and has been directly demonstrated by the experiments described above. The results of Mayr (1950), who showed that the removal of the antennae from the females of both species significantly reduced their sexual receptivity but made them equally receptive to pseudoobscura males, also support this conclusion.

SUMMARY

Sexual isolation between the closely related species, *D. persimilis* and *D. pseudoobscura*, was studied by means of male multiple "choice" tests, female multiple "choice" tests, and multiple "choice" experiments involving the males and females of both species. The female "choice" and multiple "choice" experiments gave essentially the same result, a much higher degree of sexual isolation ($I = 0.069$ and $I = 0.058$), than the male "choice" experiments ($I = 0.457$). These results indicate that the high degree of sexual isolation between these two species is primarily due to the behavior of the females.

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Vol. LXXXVIII, No. 839 The American Naturalist March–April, 1954

CORN GRASS: I. CORN GRASS AS A PROTOTYPE OR A FALSE PROGENITOR OF MAIZE

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It has been postulated that corn grass, a macromutant of maize, represents a reversion to an ancestral type of maize (Singleton, 1951). There is another distinct possibility that while corn grass may not be in itself the ancestral type of maize, it is capable of revealing in a highly accentuated form many of the characteristics of the primitive grass from which maize and its relatives have descended.

Corn grass is a type of maize which is unique not only as one of the few known macromutants in plants (Goldschmidt, 1952), but also because of its many morphological pecularities. The characteristics of this macromutant result from a change in the degree of development of both functional and rudimentary parts inherent to normal maize (Galinat, 1953). Some of the resulting features are scattered in a less pronounced form among the various races of maize. Other traits of corn grass are not typical for any of the races of maize but may reflect characteristics of other members of the maize tribe, the Maydeae.

It is improbable that corn grass is a hybrid between maize and either teosinte or Tripsacum. This is borne out by the history of corn grass and by the fact that its wide array of characteristics is inherited as a single dominant gene. Apparently no gross chromosome abnormalities are associated with the *Cg* (corn grass) gene since the crossover values for other genes (d_1 and lg_2) closely associated with the *Cg* gene are unaffected (Galinat, 1952). It is of course possible that the *Cg* gene may have originated following the duplication of a small region of the chromosome comparable to that which produces bar-eye in Drosophila.

Corn grass is highly susceptible to genetic modification. The early selections for weak expression of the corn grass gene were similar to the teopod mutant while later selections approached normal maize (Galinat, 1953). The original grass-like stocks of corn grass showed a far greater divergence from normal maize than did the teopod mutant described by Weatherwax (1929). The *Cg* and *Tp* (teopod) genes are non-allelic. The *Cg* gene is located in the chromosome-3 linkage group (Galinat, 1952) while the *Tp* gene is located in the chromosome-7 linkage group (Lindstrom, 1925).

The kernels of corn grass are enclosed by rachis bracts or spathes rather than by the elongated rachilla bracts or glumes characteristic of archaeological pod maize and of those Maydeae most closely related to

[1]This paper represents a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, Departments of Agronomy and Genetics, University of Wisconsin.

maize. Rudimentary spathes are present in typical modern maize, as in most other grasses, where they are visible in the young ear as ridges subtending the spikelet primordia. Archaeological evidence of the early existence of spathe-bearing maize is almost lacking. The exception is a discovery by Cutler (1951) of three ears estimated to be 4000 years old which have subsequently been identified as either teopod or minus modified corn grass. These ears are probably from teopod maize since the former is known to be a frequently recurring mutant of maize.

The kernels of *Coix*, an Oriental member of the Maydeae, are enclosed by glumes as well as subtending spathes (Weatherwax, 1926). When this combination of spathes and long glumes was synthesized in one experimental hybrid of pod maize and corn grass, the results appeared to be of a monstrous nature (Galinat, 1953). Thus, the corn-grass phenotype bypasses the pod characteristic of primitive maize and substitutes the spathe feature which may be characteristic of a more remote ancestor.

Proliferation of the inflorescence, an occasional abnormality in the grasses and sedges, serves a useful purpose for plants of the grass-like extreme of corn grass. The weight of the propagules or tassel plantlets from these leafy inflorescences often causes the slender culms to fall prostrate and function as stolons with numerous vegetative offshoots (Galinat and Andrew, 1953). Although such a type of stolon-producing corn grass could by synthesized for ability to survive as an escape, the plant might in no way resemble that of wild maize and would be just another example of a false progenitor of a modern species.

The characteristics of corn grass appear to be in the nature of general inherent reflections from all the Maydeae rather than from any immediate wild ancestor of modern maize. It has been commonly observed that the series of grass-like features which are listed for corn grass (table 1) are essentially a single characteristic since their counterparts from normal maize tend to be modified as a unit. Since the length of internode and sex expression may be interrelated in the tillers of normal maize (Werth, 1922), the type of sexual development and length of internode may, for this comparison, also be considered as a single characteristic.

There are several inherited changes of maize which have almost as extensive morphological effects as the corn grass and teopod mutations. The mutation of tunicate or pod maize to normal described by Mangelsdorf (1948) might also be characterized as a macromutation. Mangelsdorf (1950) has suggested that the *Tu* (tunicate) gene causes the production of virtually all of the characteristics that one might expect to find in the ancestral form of maize. Plants bearing the recessive ramosa genes (ra_1 or ra_2) have inflorescences which branch freely and resemble a sorghum head (Gernert, 1912). The recessive genes for dwarf plant (d_1 to d_7) are responsible for short internodes, broad thick leaves and stamens on the ear. These "macromutations" as well as many others are listed by Emerson, Beadle and Fraser (1935).

TABLE 1

LISTING OF FIVE OF THE MORE CONSISTENT CHARACTERISTICS OF CORN GRASS TOGETHER WITH THEIR COUNTERPART IN THE OTHER MAYDEAE

Corn grass characteristics	Counterpart in other Maydeae
1. Grass-like features a. Numerous tillers b. Many small ears c. Slender culms d. Narrow leaves e. Slender pistillate rachises	All of Maydeae except typical maize.
2. Tripsacum-like sex-growth relations. a. Short internodes b. Basal portions of terminal inflorescence pistillate	All of Maydeae except main culm of typical maize.
3. Single pistillate spikelets	All of Maydeae except typical maize.
4. Terminal inflorescence with few branches	Oriental Maydeae and some species of *Tripsacum*.
5. Spathes subtending spikelets	*Coix* (Oriental Maydeae)

The pod-pop character of primitive maize ears was established when comparisons of glume and kernel type were made with the archaeological remains of maize from Bat Cave, New Mexico (Mangelsdorf and Smith, 1949). The oldest ears from Bat Cave are suggestive of a small-eared type of modern pod popcorn which is neither grass-like nor perennial.

It has been found that the hybrids resulting from crosses between certain small-eared varieties of popcorn and minus modified corn grass have the original grass-like features of this macromutant. Such was not the case when varieties of dent, flint and sweet maize were used in similar crosses (Galinat, 1953 and unpub.). Although corn grass is probably not the ancestral type of maize, the results of studies of its hybrids may still be of value in locating relic germplasm characteristic of the primitive grass from which maize and its relatives have descended.

ACKNOWLEDGMENT

The writer expresses his gratitude to Drs. R. H. Andrew, D. C. Cooper, and E. L. Fisk for their guidance and interest throughout the investigation.

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Vol. LXXXVIII, No. 839 The American Naturalist March-April, 1954

A CYTOLOGICAL ANALYSIS OF IRRADIATED POPULATIONS*

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Wallace (1950, 1951) and Wallace and King (1951, 1952) have reported recently on the genetic analyses of irradiated populations of *Drosophila melanogaster.* The following note presents some data concerning the frequencies of chromosomal aberrations within several of these same experimental populations.

The populations analyzed were those designated No. 1, No. 5, and No. 6. Populations 1 and 6 are large populations that contain approximately 10,000 adult flies at all times; population 5 is a small population that frequently has fewer than 1,000 individuals. The initial parents of population 1 were exposed to X-rays; males received 7,000 r and females received 1,000 r. This population has received no subsequent irradiation. Populations 5 and 6 are exposed continuously to radium "bombs"; each receives about 2,000 r of gamma-radiation each generation (5 r each hour). At the time these cytological analyses were made population 1 was about 100 generations old while populations 5 and 6 were about 20 generations younger.

Larvae developing from egg samples removed from the populations were used for the salivary-gland studies. Cups of cornmeal-agar-molasses medium were exposed to the populations for 24 hours after which they were removed. The eggs laid during the time of exposure were subdivided among several 1/2 pint culture bottles containing medium enriched with brewer's yeast. These cultures were kept at 15°-18°. During the first and second larval instars, additional fresh yeast was added to the cultures as a thick solution. During the third instar, granulated yeast was added. The cytological examinations of salivary gland chromosomes were made with aceto-orcein temporary smears.

The frequencies of inversions and translocations in the three populations are given in table 1. In population 1 two larvae of the 121 examined were heterozygous for inversions; these inversions were not identified. During generation 56 (44 generations before the present study) an analysis of 52 larvae revealed that 13.5 per cent of these were heterozygous for an inversion in the right arm of chromosome 2. This inversion has apparently been eliminated during the time following this early study. During the same interval there has been an elimination of second chromosome lethals; to what extent these represent two manifestations of the same process is unknown.

*The work reported here was done in connection with Contract No. AT-(30-1)-557, U. S. Atomic Energy Commission.

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TABLE 1

FREQUENCIES OF INVERSIONS AND TRANSLOCATIONS IN IRRADIATED POPULATIONS OF D. MELANOGASTER. N = NUMBER OF LARVAE EXAMINED; 2N = NUMBER OF HAPLOID SETS.

Population	N	2N	Per cent inv.	Per cent trans.
1	121	242	0.8	0
5	100	200	13.5	1.0
6	41	82	4.9*	1.2

*4.9 per cent of non-Plum inversions; frequency of Plum was 19.5 per cent.

Of the three populations studied, number 5 had the highest frequency of individuals carrying newly formed chromosomal aberrations; 14.5 per cent of all larvae examined carried either inversions or translocations. Of the inversion-heterozygotes observed in this population, two-thirds (18/27) carried identical inversions—a terminal inversion on the left arm of chromosome 3. It is impossible to tell from a single examination whether this inversion has a high frequency as a result of chance (genetic drift) or selection. It is most probable that the cause is selection because, if this one inversion is eliminated from the data, the total inversions in this population is the same as that in population 6. The chief effect of genetic drift on frequencies of individual inversions should be a distribution of their frequencies with an abnormally large variance; the mean frequency of all inversions should be affected only secondarily through its relation to the total variance. The frequency of new inversions in population 5 is more than twice that in population 6.

With the exception of the terminal inversion in population 5, the frequencies of inversions and translocations in populations 5 and 6 are very

TABLE 2

LOCATION OF INVERSIONS FOUND WITHIN POPULATION 5.

Chromosome	Cases observed	Position of breaks
2 R	2	46F–51A
2 R	1	46F–50C
2 R	1	44C–46F
3 L	1	68B–76C
3 L	18	61A–65A
3 R	1	90A–93A
unidentified	3	....

similar. There exists some evidence among these inversions that genetic drift is operating within population 5: Of the six identified inversions from population 5, 2 or, perhaps, 3 of them were identical; each of the 4 inversions identified in population 6 differed from the others. Thus, both selection and drift appear to be operating within the small population.

In addition to newly induced inversions, population 6 also contains a high frequency of In(2LR)Pm, a pericentric inversion associated with the domi-

nant mutation *Plum*. From phenotypic observations it was already known that this inversion had established a stable, polymorphic system within this population. The frequencies of *Plum* estimated by the two types of observations do not differ: the estimate based on phenotypes was 15 per cent (2,718 flies observed); the 95 per cent confidence interval for the cytological observation extends from 12 per cent to 28 per cent. Since *Plum* is lethal when homozygous, the stable equilibrium must result from a selective advantage of *Plum* heterozygotes over the wild-type flies of population 6.

Finally, it is of some interest to compare the frequencies of inversions with the frequencies of translocations. Excluding the two inversions for which there is reason to suspect a positive selection in the heterozygous condition (terminal inversion in population 5, Plum in population 6), we can combine the frequencies of the remaining inversions for the two populations. The combined frequency is 4.6 per cent (13/282). The combined frequency of translocations is 1.1 per cent (3/282). Inversions, then, have an overall frequency about 4 times as large as that of translocations. In general, translocations are exposed to a selective disadvantage of 0.5 in the heterozygous condition; this is a result of the semi-sterility of such heterozygous individuals. The frequency of translocations observed, then, should be about twice their rate of formation by the irradiation. In *D. melanogaster* translocations and inversions arise with approximately equal frequency after irradiation (Lea, 1947). Therefore, if inversions accumulate in populations to a frequency four times as large as that of translocations, the selection against inversion heterozygotes must be only one-fourth as large as that of translocations or approximately 0.125 on the average. These figures, however, must be regarded as approximations because of the small samples involved.

ACKNOWLEDGEMENTS

My stay in the United States was made possible by a Fulbright grant for which I wish to express my appreciation. I wish also to thank Dr. M. Demerec for the facilities put at my disposal at the Biological Laboratory and Dr. Bruce Wallace both for his helpful advise and for giving me the opportunity to work on this problem.

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Vol. LXXXVIII, No. 839 The American Naturalist March–April, 1954

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

MUTATION TESTS WITH A FORMALDEHYDE—HYDROGEN PEROXIDE MIXTURE IN DROSOPHILA.

The mutagenic effect of mixtures of hydrogen peroxide and formaldehyde was first shown in Neurospora by Dickey, Cleland and Lotz.[1] The active principle in this mixture was thought to be an organic peroxide and its subsequent decomposition by which free radicals are produced. Similar results were obtained by Jensen, Kirk, Kølmark and Westergaard.[2] Moreover, these authors proved that in Neurospora, treatment of conidial spores with a mixture of hydrogen peroxide and formaldehyde gives a much higher mutation rate than the application of formaldehyde alone. To explain the mutagenic effects of formaldehyde they suggest an autoxidation sequence which also leads to the formation of free radicals. The fact that pretreatment of formaldehyde with ultra violet light and the addition of hydrogen peroxide increase its mutagenic activity seem to support this explanation.

Auerbach[3] found that formaldehyde solutions when injected into Drosophila males produce mutations. We were therefore interested to see whether a mixture of formaldehyde and hydrogen peroxide when injected into Drosophila would also cause a higher mutation rate than formaldehyde alone. Such a mixture has already been applied in feeding experiments where it was ineffective (Auerbach unpublished). But as the possibility has to be considered that formaldehyde food and formaldehyde solution act in different ways (Auerbach[4]), injection of the mixture seemed worth trying.

The compounds were injected in the abdominal cavity of two-day old Oregon-K males. The offspring of these males were tested for sex-linked lethals by the Muller-5 technique. No controls were used, but the spontaneous mutation rate in the Oregon-K stock hardly ever exceeds 0.3%. The sensitivity pattern in the testis of the treated males could be studied by mating them to three fresh virgin females at time intervals of three days during the first twelve days after treatment (cf. also Auerbach[4]).

The formaldehyde used throughout the experiments was a 36% commercial preparation (Analar, B. D. H.). All solutions were made with 0.75% sodium chloride in distilled water. The proportion of formaldehyde and hydrogen peroxide in the mixture was the same as the one applied successfully to Neurospora spores by Jensen et al. The formaldehyde solution was made up to contain 0.067 Mols/litre. The formaldehyde—hydrogen peroxide solution contained the same concentration of formaldehyde and in addition

0.044 Mols hydorgen peroxide per litre. Additional experiments with other concentrations were made. To reduce possible chemical alterations in the mixture it was freshly prepared before each injection series and used within seven minutes of preparation.

The results of two experiments are summarized in the accompanying table. They show that there is no difference between the frequency of sex-

TABLE 1.

THE FREQUENCY OF SEX-LINKED LETHALS IN SUCCESSIVE BROODS FROM MALES WHICH HAD BEEN INJECTED WITH MIXTURES OF FORMALDEHYDE AND HYDROGEN PEROXIDE AND FORMALDEHYDE ONLY

Expt.	Concentration	Days after treatment	No. of tested males	No. of tested spermatozoa	No. of lethals	Percentage lethals
I	0.067 mol. CH_2O + 0.044 mol. H_2O_2	0–3	23	517	11	2.2
		3–6	10	260	2	0.8
		6–9	4	116	....	....
		9–12	10	190	3	1.5
		12–15	5	136	....	....
	0.067 mol. CH_2O	0–3	26	635	18	2.9
		3–6	8	206	2	1.0
		6–9	4	191	....	....
		9–12	17	345	7*	2.0
		12–15	15	443	....	....
II	0.067 mol. CH_2O + 0.044 mol. H_2O_2	0–3	25	843	43	5.1
	0.067 mol. CH_2O	0–3	±20	450	22	4.9
	0.050 mol. CH_2O + 0.033 mol. H_2O_2	0–3	27	883	7	0.9

*Three of these lethals appeared as a bunch derived from the same male.

linked lethals induced by injection of the mixture and of formaldehyde alone. Considering the mutation rates in the different broods a similar sensitivity of corresponding stages of germ cell development will be observed. The reason for the difference in the mutation rates between the two experiments is not apparent.

The inconsistency of our results with those obtained in Neurospora is probably due to a high catalase activity in the fly, responsible for a rapid breakdown of the hydrogen peroxide. This assumption was confirmed by a sensitive test for the presence of hydrogen peroxide. Immediately after having been injected with the hydrogen peroxide mixture the fly was

squashed in a drop of titaniun sulphate, (one per cent in four normal hydrochloric acid). No orange colour, characteristic for the presence of hydrogen peroxide could be observed then. Similarly it could be shown that nydrogen peroxide, if brought into contact with the interior of a squashed fly is decomposed immediately. In this respect no differences were found between males and females. Decomposition of the hydrogen peroxide mixture before the injections does not occur, however, within one hour which is much longer than the interval between preparation and injection in the present experiment. A presumptive indication of high catalase concentration in adult flies has also been reported by Di Paolo.[5]

The similar mutations rates induced by the mixture and the formaldehyde suggest that the mutations observed after injection of the mixture must be ascribed to the mutagenecity of the formaldehyde only. Oxidation of the formaldehyde to formic acid does not seem likely. Though formic acid acts as a mutagen in bacteria, its effectiveness is much less than that of formaldehyde (Demerec, Bertani and Flint[6]). In feeding experiments of Drosophila larvae it does not produce mutations (Alderson, unpublished).

To see whether the similar mutation rates of formaldehyde and the mixture are due to a higher toxicity of the latter, leading to selective killing of the affected germ cells, and experiment with slightly lower concentrations was carried out (table, bottom line). The conspicuous drop of the mutation rate does not confirm this assumption. It demonstrates at the same time the limited concentration range that can be used to observe the mutagenic effects of these compounds; preliminary experiments showed that a mixture based on 0.084 mol. formaldehyde is too toxic.

Jensen et al have put forward the idea that formaldehyde might act through the formation of peroxides. Auerbach[4] has considered that the same might apply to formaldehyde when injected into Drosophila adults. Further evidence concerning this question cannot be obtained from the experiments recorded here. Experiments are now under way on the mutagenic properties of the pure peroxide formed by combining formaldehyde and hydrogen peroxide, and on the influence of catalase inhibitors.

Considering the pattern of sensitivity it can be seen that the highest mutation rates were observed in the first three days after treatment, that is in mature sperm. After a decrease of the mutation rate in the second and third broods, it increases again in the fourth brood. A similar rise in later broods has been observed by Auerbach.[3] The three lethals in the fourth brood after formaldehyde injection were shown to be identical both by localization and phenotypic effect. Their occurrence might point to a coincidence of the second sensitive period with spermatogonial stages. Auerbach[4] found maximum sensitivity for formaldehyde feeding during the auxocyte stage. It cannot be decided here, however, whether injected formaldehyde and formaldehyde fed to the larvae act on different young stages in germ cell development.

This investigation was carried out under a British Council Scholarship. The author wants to express his gratitude to Dr. C. Auerbach for suggesting

the problem and her stimulating interest throughout the work, and to Professor C. H. Waddington for the hospitality received in his Institute.

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TRIADS OF OVERLAPPING INVERSIONS IN *DROSOPHILA ROBUSTA*

The recent paper of Wallace[1] in this journal is most interesting. He sets forth the hypothesis that three overlapping inversions create difficulties for coadaptation of pairs of them and, therefore, all three should rarely be found together. This hypothesis is supported by the nearly complete absence of populations of *D. pseudoobscura* in which all three members of such triads are present in high frequencies. Our greater familiarity with *D. robusta*, however, leads us to believe that, contrary to his suggestion, the data from this species do not support the hypothesis. The following facts have led us to this conclusion.

1. In addition to those noted by Wallace, the following population frequencies for the triads listed in his Table 2 (page 347) have been reported[2,3]:

XL-XL1-XL2: 75-6-19; 58-13-29; 43-20-38; 34-32-35; 26-41-33; 14-42-44; 42-49-9.

2L-2L2-2L3: 27-17-19; 24-16-26; 19-16-45; 19-7-51; 22-8-54; 20-5-66; 27-18-33.

2L-2L1-2L3: 20-8-66; 22-16-54; 19-24-51; 19-20-45; 24-34-26; 27-37-19; 27-22-33.

When these data are combined with the ones quoted in his Table 2, the reported populations may be grouped as follows with respect to the hypotheses:

Triad	Supporting: (at least one arrangement < 5%)	Possibly supporting: (one arrangement with frequency of 5–10%)	Contrary: (All three > 10%)
XL-XL1-XL2	6	3	5
XL-2L2-2L3	5	5	5
2L-2L1-2L3	5	2	7
Total	16	10	17

An argument may be made, supported by such data as those reported in our seasonal studies[4,5], that even rare arrangements appear to be in stable, hence probably adaptive, equilibrium in the populations. However the group labelled "possibly supporting" above is interpreted, the data show that many populations of *D. robusta* contain all three members of a triad in substantial frequency. Additional data for all three triads have been reported recently[6] but fail to support Wallace's hypothesis.

2. Even in those instances that seem to fit the hypothesis, it is possible that the apparent absence or rarity of one member of the triad is not a function of the cytological problem indicated but is instead a function of geographical and ecological distribution. Thus, the frequency of arrangement 2L-3 increases with latitude[7] and altitude[8] and, in some localities, increases during the colder months of the year and decreases in the warmer

months.[3,6] These facts suggest that the climate plays an important part in determining whether this arrangement becomes established in a locality in substantial frequency.

Although the data from populations of *D. robusta* do not support Wallace's thesis, it should be mentioned that they do not contradict it. The arrangements concerned are broadly overlapping and, as Wallace points out, stable coadapted complexes are possible even for triads of overlapping inversions. Above all, there is indicated the need for additional quantitative studies, particularly with species having inversions which barely overlap one another.

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FURTHER NOTES ON ANNELID GROWTH

In the November-December last issue of THE AMERICAN NATURALIST Hyman[1] claims that she was misquoted by the present writer in an earlier issue (Moment, 1953)[2]. For the record it should be said that the statement in question, viz., that "any oligochaete" will grow an indefinite number of segments posteriorly, is to be found on page 522 line 15 of Hyman's 1940[3] review of regeneration in annelids. On this point the present writer simply said as a matter fact that it was the generally accepted belief that earthworms continue to grow indefinitely by segment formation at their hind ends, and cited the review by Hyman to substantiate the statement that such was the view accepted by authorities in this field. By adding "Only Sun and Pratt, (1931) had published views at variance with those accepted by Hyman" and pointing out that even their conclusions had been questioned by at least one competent oligochaetologist, the present writer had hoped to make it clear that before his own work on this problem (Moment, 1950)[4] there had been no good reason to doubt the common view.

The other two points raised by Hyman are more interesting and important because they are general criticisms of the particular form of bioelectric and anatomical theory of growth regulation under discussion. Perhaps because the species of worm on which most of the work has been done possesses a fixed number of segments, it has evidently seemed to Hyman and possibly to others that the theory itself carried an implication that a finite number of segments was common to annelids generally, and that the theory applied only to organisms having such a fixed segment number. Actually neither of these views is correct. It was for this reason that care was taken in earlier papers to specify earthworms rather than annelids generally when talking about a definitive segment number. A recent paper (Moment, 1953)[5], which establishes beyond all possible doubt that in *Eisenia foetida* there is a definitive number of segments attained before the worms emerge from the egg cocoon, also points out explicitly that such facts should not be taken to mean that all earthworms, especially those belonging to families other than the Lumbricidae, possess a fixed number of segments. The work of Gates[6] on an Oriental earthworm was cited as a case where segment formation is known to continue, if not indefinitely, at least for a long time.

Furthermore, the theory does not presuppose that an animal must have a fixed number of segments. Rather it is based on what used to be called "Morgan's law" that the rate of regeneration becomes progressively slower as the site of regeneration becomes more and more distal. Put briefly, it maintains that there is a causal relationship between this gradual decline in rate of proliferation and the concomitant rise in electrical potential at the more distal anatomical levels. Consequently this is not a theory that applies only out at the extreme tip of a worm when and if proliferation comes to a complete standstill. On the contrary, it applies at all anatomical levels. This feature of the theory was fully discussed in a paper (Moment, 1953)[7] on body level, temperature, and nutrition in relation to rate of

regeneration; but "Morgan's law" was mentioned several times and growth was described as slowing to an asymptote in the paper referred to by Hyman. The peculiar fact about common earthworms that renders them favorable experimental material is that in them the asymptote actually reaches zero, and that it does this so early in development. Perhaps it should be added that Morgan's so-called law of regeneration is not a phenomenon of very limited applicability. It has been found to hold for some 30 species distributed through seven phyla.

Hyman also suggests that it is "meaningless" to measure potential from one end of a worm to the other, as has been done by the writer and other investigators, because it can be shown that both ends are electropositive to the middle. Conceivably it may turn out that bioelectric potentials are epiphenomena without morphogenetic meaning. But the fact that both ends are electropositive to the middle neither proves nor disproves it. Indeed, the fact that distal regions, whether anterior or posterior, are electropositive to proximal ones correlates with Morgan's law that proliferation is slower in more distal regions whether anterior or posterior and with what has been said above to the effect that the more distal the anatomical level, the more electropositive it becomes and the more limited the growth rate.

Technically it seemed easier to get repeatable results when the potential was measured from one end to the other of a worm than when one electrode was on the middle of the worm. However, it is to be remembered that the proliferating hind end is clearly positive by either measurement and simple arithmetical considerations show that either way there would be a similar increase in potential with growth. Consequently the argument regarding the effect of an increasing potential at the posterior growing zone remains unchanged.

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AN IMPROVED TECHNIQUE FOR DOMINANT LETHAL STUDIES IN DROSOPHILA.*

Since Lüning[1] reported an effect of age on the production of dominant lethal mutations in the irradiated sperm of *Drosophila melanogaster*, it has become increasingly important to know the exact age of the sperm in relation to the time of irradiation when carrying out genetic studies. The techniques described by Lüning[1], Demerec and Fano[2], and Baker and Vonhalle[3] require much time and effort in collecting eggs of various ages for dominant lethal studies. A method which requires less time is described below.

Inseminated females are placed in a specially constructed glass funnel. This funnel which is 85 mm in diameter does not taper for the first 25 mm. It then tapers to a diameter of 10 mm. A piece of Dacron netting, Dacron marquisette having a 54 × 34 count (Wellington Sears Co. Style 1848), is stretched over the 85 mm opening and held in place by a heavy rubber band. The other end, 10 mm opening, is closed with a cotton plug. The openings in the Dacron netting are too small to permit adult Drosophila to pass through, but large enough to pass eggs. This funnel is inverted on a petri dish containing standard Drosophila food which has been heavily seeded with yeast 6 hours previously. A slight amount of pressure is applied to the funnel to assure that the netting is in direct contact with the surface of the food. Then the females oviposite the eggs through the netting on to the food. After a specified time, the females are transferred to new medium by simply picking up the funnel and placing on a fresh petri dish of food. Because of the minute size and the extremely smooth surface of the Dacron thread, in no cases studied have we observed eggs sticking to the netting.

The eggs are then counted on the surface of the medium using an 10X dissecting microscope. We have found that counting of eggs on such a large surface can be facilitated by adding charcoal lines to the surface; this is accomplished by dipping 1 cm sq netting in powdered charcoal and pressing it lightly against the surface of the medium. The petri dish is then covered and incubated for 24–48 hours at 25°C. The number of eggs that have not hatched at the end of this period are counted and recorded as dominant lethals. If the larvae from the hatched eggs are to be raised to adult stages for further genetic studies, the medium is quartered and the pie-shaped pieces of medium are placed in standard bottles of medium.

LITERATURE CITED

[1]Lüning, K. G., 1952, X-ray induced dominant lethals in different stages of spermatogenesis in Drosophila. Hereditas 39:91–107.

[2]Demerec, M. and U. Fano, 1944, Frequency of dominant lethals induced by radiation in sperms of *Drosophila melanogaster*. Genetics 29:348–360.

*Contribution No. 547, Department of Zoology, Indiana University, Bloomington, Indiana. Work has been supported by a grant to Dr. H. J. Muller from the United States Atomic Energy Commission (Contract AT(11–1)–195).

[3]Baker, W. E. and E. S. Von Halle, 1953, The basis of the oxygen effect on X-irradiated Drosophila sperm. Proc. Nat. Acad. Sci. 39:152-161.

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ZOOLOGY DEPARTMENT
INDIANA UNIVERSITY
BLOOMINGTON, INDIANA
February 15, 1954

THE AMERICAN SOCIETY OF NATURALISTS

SECRETARY'S REPORT, 1953

The annual business meeting of the Society was held in Boston on December 28, 1953, in connection with the AAAS, with Vice-President Alexander Hollaender presiding in the enforced absence of the President, L. J. Stadler, due to illness. A motion was made and unanimously passed to hold the next meeting with the AIBS on the campus of the University of Florida at Gainesville, September 5–9, 1954. It was also suggested that a symposium be arranged for the December, 1954, meeting of the AAAS to be held at the University of California in Berkeley.

The report of the Nominating Committee, Dwight Minnich, chairman, L. R. Dice, and D. R. Goddard, was presented by the Secretary. With no further nominations from the floor the following officers were unanimously elected: President for 1954, Dr. Milislav Demerec, Director of the Carnegie Institution at Cold Spring Harbor, Long Island, N. Y.; Vice-President for 1954, Dr. Ralph W. Chaney, Professor and Curator of the Museum of Paleontology, University of California, Berkeley; Treasurer, 1954 through 1956, Dr. Carl P. Swanson, Professor of Biology, the Johns Hopkins University, Baltimore; and three members of the Editorial Board of *The American Naturalist*, 1954 through 1956, Dr. A. S. Foster, Professor of Botany, University of California, Berkeley; Dr. Jack Schultz, Head of the Department of Genetics and Cytochemistry, the Institute for Cancer Research and the Lankenau Hospital Research Institute, Philadelphia; and Dr. G. L. Stebbins, Professor of Genetics, the University of California, Davis, California.

The reports of the Treasurer, D. F. Poulson, and of the Secretary were read and approved. C. P. Swanson and W. K. Baker served on the auditing committee, and Dr. Swanson reported the treasurer's books and accounts in order. A vote of appreciation was given to Dr. Alfred Romer, who served as local representative on arrangements for the Boston meeting.

Dr. Poulson, the retiring Treasurer, pointed out that unless dues were raised this year the Society would soon be in the red. He moved that the annual dues be raised from $5.00 to $6.00. After some discussion this motion was unanimously passed.

Individuals nominated by members of the Society, and approved by the Executive Committee, were elected to membership. A list of those who have accepted membership to date is appended to this report. A supplementary list of others accepting membership will be published at a later date.

At the Boston meeting on the afternoon of December 28, in the Ballroom of the Sheraton Plaza Hotel, Dr. Harold H. Plough presided at a Symposium on "Some Biological Effects of Radiation from Nuclear Detonations." This symposium was arranged by Vice-President Hollaender, and co-

sponsored by the Genetics Society of America. Subjects and speakers were as follows:

Chromosomal Breakage in Tradescantia, Alan D. Conger
The Production of Chromosome Rearrangements in Drosophila, E. B. Lewis
Visible and Lethal Mutations in Drosophila, George H. Mickey
The Effect of Neutrons on Thymic and Circulating Lymphocytes of the Mouse, Robert Carter, V. P. Bond, James T. Brennan, and E. P. Cronkite (Dr. Carter presenting the paper)
Genetic and Developmental Effects on Mice, William L. Russell

These symposium papers will be published in *The American Naturalist.*

On Tuesday, December 29, a Symposium on "Physics in Biology," arranged by R. S. Bear for Section B of the AAAS (Section on Physics) and cosponsored by the American Society of Naturalists was presented. At the morning session, with R. S. Bear presiding, the following papers were presented:

The Reversal of Biological Effects of Radiation, Alexander Hollaender
The Physics of Self-duplication, Ernest C. Pollard
Three-dimensional Features of Protein Structure, Barbara W. Low
Development and Present Status of Electron Microscopy in Biological Research, Cecil E. Hall

At the afternoon session Dr. Hollaender presided and these papers were given:

Physics of Muscular Fatigue, Kurt S. Lion
The Physics of Circulatory Mixing, C. W. Sheppard
The Use of Isotopes in the Study of Man, Arthur K. Solomon
In Vivo Radioisotope Measurement, Gordon L. Brownell
Contributions of Spectroscopy to the Study of Biologically Significant Trace Constituents, Bert L. Vallee

The Naturalists' Dinner was held on Wednesday evening, December 30, at the Hotel Copley Square, with about sixty in attendance. In the absence of President Stadler, his Presidential Address on "The Gene" was read by Dr. Bentley Glass. The address was a masterful presentation of the subject, with a clear analysis of the known facts, discovered through experiment and observation, and of theoretical speculation, awaiting further experiment for verification. On a motion by Dr. Hollaender, who presided at the dinner meeting, the members sent a telegram of appreciation and best wishes to Dr. Stadler.

The Executive Committee for the year 1954 consists of M. Demerec, Chairman, R. W. Chaney, Alexander Hollaender, P. C. Mangelsdorff, W. P. Spencer, L. J. Stadler, C. P. Swanson, and Sewall Wright. Representatives on the AAAS Council for 1954 are R. K. Burns and H. H. Plough, and Representative on the AIBS Governing Board for 1954 is C. G. Huff.

The following deaths not previously reported have occurred: J. W. Buchanan, E. C. Case, E. G. Conklin, A. L. Hagedoorn, C. L. Huskins, Harold Kirby, and H. J. Van Cleave. The year 1953 closed with an active membership of approximately 470 and an emeritus membership of about 175. It should be understood that active members, on retirement from their professional posts, may become emeritus members, with exemption from dues.

Emeritus members may continue to subscribe to *The American Naturalist* for the $3.50 subscription rate paid by the Society for each active member. Request for emeritus status should be sent to the Secretary.

Membership in any organization implies some responsibilities as well as privileges. Members can best support the program of the Naturalists Society by (a) attending the annual symposia and other functions arranged by the Society; (b) attending and taking an active part in the annual business meetings; (c) supporting *The American Naturalist* by submitting papers for publication and calling the attention of others, whether they be members or not, to this journal as an outlet for the publication of papers coming under the categories listed on the editorial page; and (d) nominating to membership individuals with a broad interest in biology and evolution, who have proven by a number of publications of high merit that they are worthy of membership.

It is to be hoped that in a very few years the active membership may reach the 600 mark mentioned in the Report of the Committee on Policy, submitted some twenty-five years ago. There are undoubtedly this many biologists in the country, whose productive scholarship and breadth of interest make them eligible. As the former Secretary, Dr. Glass, stated in his report for 1952 "there has been no alteration of the standards required for election to membership in the Society." This will be evident to anyone who takes the trouble to examine new membership lists of the past forty years, with consideration of the published work of the individuals at the time of election.

In closing this report the Secretary asks your cooperation in the matters mentioned above, and hopes that many, if not most, of you may be able to attend the Gainesville or Berkeley meetings.

Respectfully submitted,
WARREN P. SPENCER
Secretary

COLLEGE OF WOOSTER
WOOSTER, OHIO
February 7, 1954

NEW MEMBERS, 1953

Mark H. Adams, New York University, College of Medicine
Dean Amadon, American Museum of Natural History
Norman G. Anderson, Oak Ridge National Laboratory
Ralph V. Bangham, College of Wooster, Ohio
Ludwig von Bertalanffy, University of Ottawa
Robert Briggs, Institute for Cancer Research, Philadelphia
Vernon Bryson, Biological Laboratory, Cold Spring Harbor, N. Y.
Edward C. Cantino, University of Pennsylvania
Barry Commoner, Washington University, St. Louis
A. H. Doermann, University of Rochester
Eldon J. Gardner, Utah State Agricultural College, Logan, Utah

Robert P. Geckler, Oak Ridge National Laboratory
Aloha M. Hannah, University of California
Theodore S. Hauschka, Institute for Cancer Research, Philadelphia
Robert L. Hulbary, State University of Iowa
John S. Kirby-Smith, Oak Ridge National Laboratory
Harlan Lewis, University of California, Los Angeles
Ralph W. Lewis, Michigan State College
R. Barclay McGhee, Rockefeller Institute for Medical Research
James V. Neel, University of Michigan
David D. Perkins, Stanford University
Colin S. Pittendrigh, Princeton University
Ernest Pollard, Yale University
John R. Reeder, Yale University
Charles L. Remington, Yale University
Bobb Schaeffer, American Museum of Natural History
John R. Totter, Oak Ridge National Laboratory
Marshall R. Wheeler, University of Texas
Karl M. Wilbur, Duke University
Stephen Zamenhof, Columbia University, College of Physicians and Surgeons

REPORT OF TREASURER, 1953.

American Society of Naturalists.

The Society's balance at the beginning of 1953 was $1729.81. As pointed out in the previous report (1952) the size of this balance is partly because the Society did not receive billing for its 1952 dues to the A.I.B.S. until the present year. These amounted to $243.00 as shown in the statement of expenditures. Receipts for the year for dues and subscriptions to *The American Naturalist* amounted to $2216.50 of which $1676.50 were paid to The Science Press for 463 subscriptions for 1953 and 16 for the years 1951 and 1952. The contribution toward the editorial expenses of *The American Naturalist* for 1953 was $300.00. Together the payment for subscriptions and the editorial expenses come to $1976.50. When this is deducted from the year's receipts the remainder is $240.00 which is less than last year's dues to the A.I.B.S. and a good deal less than this year's dues which were $320.50. Other expenditures this year have been comparatively small. Total expenditures (exclusive of the Secretary's expenses for travel to this meeting which will have to be paid next year) came to $2615.36. The Excess of Expenditures over Receipts amounted to $398.86. While the balance as of Dec. 27, 1953 is $1330.95, it is perfectly clear that this balance will have vanished within the next three years and that the Society will be insolvent unless the dues are increased.

It is quite clear that the membership as a whole has supported the arrangement with *The American Naturalist*, the number of subscriptions having increased from 412 in 1951 to 463 in 1953. There are still about 20 members who owe dues for the past two years and 9 who owe for three years. The latter will be dropped. It is hoped that the former will pay up promptly.

Respectfully submitted,
D. F. POULSON
Treasurer

Statement of Receipts and Expenditures: Jan. 1–Dec. 27, 1953.

Balance: Jan. 1, 1953, $1,888.01 in Second Nat. Bank less outstanding checks for $158.20		$1,729.81
Receipts: Dues and subscriptions to *The American Naturalist*	$2,216.50	
Expenditures: The Science Press, Lancaster, Pa. 463 subscriptions to *The American Naturalist* @$3.50 (for 1953)	1,620.50	
The Science Press, Lancaster, Pa. 16 subscriptions to *The American Naturalist*, @$3.50 (1951, 1952)	56.00	
National Academy of Sciences, A.I.B.S. dues for year 1952	243.00	
National Academy of Sciences, A.I.B.S. dues for year 1953	320.50	

Expenditures: (Cont.)	Dept. of Biology, The Johns Hopkins University, stamps, envelopes, express charges for Secretary's records	19.21	
	The Collier Printing Co., Wooster, O. Stationery etc. for new Secretary	26.89	
	L. C. Dunn, Editor, *The American Naturalist*, Society's share of editorial office expense	300.00	
	Second National Bank, returned checks	20.00	
	Osborn Zool. Lab., Yale University, Treas. expenses, stationery, stamps, mimeographing	6.02	
	Donald F. Poulson, reimbursement for cost of new file cards, rubber stamp	3.24	
	Total Expenditures	$2,615.36	
Excess of Expenditures over Receipts			398.86
Balance on Hand, December 27, 1953			$1,330.95

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

EDITORIAL OFFICE
The American Naturalist
Box 2, Schermerhorn Hall
Columbia University
New York 27, N. Y.

Cleveland, L. R., 1953. Studies on chromosomes and nuclear division. pp. 809-869, New Series, Vol. 43, Part 3. Transactions of the American Philosophical Society, Philadelphia.

Cold Spring Harbor Symposia on Quantitative Biology, Vol. XVIII, 1953, Viruses. 301 p., 97 figures, 15 plates. $8.00. Biological Laboratory, Cold Spring Harbor, New York.

Dalquest, Walter W., 1953. Mammals of the Mexican State of San Luis Potosí. 232 p. $3.50 (offset). Louisiana State University Press, Baton Rouge.

Ephrussi, Boris, 1953. Nucleo-cytoplasmic relations in micro-organisms. 127 p. ill. $3.75. Oxford University Press, New York.

The author begins with the theme that only through emphasis on the study of cytoplasmic heredity will we be able to unravel the mysteries of development and differentiation. He goes on to describe and explain a number of cases of cytoplasmic heredity in micro-organisms, the most important of which are Ephrussi's own fascinating work on respiratory mutants in yeast, the killer character in Paramecium, and the barrage effect in Podospora. These are thoroughly covered in a most lucid manner with particular emphasis on the intricate relations existing between these cytoplasmic factors and the corresponding nuclear genes involved in their expression. In the last chapter Ephrussi attempts to integrate the previously described work on cytoplasmic heredity in micro-organisms into a basis for the interpretation of differentiation in higher organisms.

Fothergill, P. G., 1953. Historical aspects of organic evolution. 427 p. $6.00. Philosophical Library, New York.

The author devotes almost two-thirds of the space to the post-Darwin period, but the choice of material is often uncritical. The author's sympathies clearly tend towards a brand of Lamarckism and mysticism.

T. D.

Gunderson, Harvey L., and James R. Beer, 1953. The mammals of Minnesota. 190 p., ill. $3.50 cloth, $2.00 paper. University of Minnesota Press, Minneapolis.

Each state, it appears, must have its own book of mammals. This one follows the formula: it has directions for catching mammals and turning them into museum specimens; a sketch of the geography of the state; and a systematic list with keys, a descriptive paragraph, and a distribution map for each species. Sometimes there are photographs (e.g., of the House Mouse, the Norway Rat and the Porcupine).

Heisenberg, W., 1953. Nuclear physics. 226 p., ill. $4.75. Philosophical Library, New York.

Hovanitz, William, 1953. Textbook of genetics. 420 p., 267 text-figures, 48 tables. $5.95. Elsevier Press, Houston and New York.

A general introductory text book for college courses with concluding chapters on applications in medicine and agriculture and in evolution; appendices on suggestions for laboratory work; and probability tables for Mendelian ratios.

Kallmann, F. J., 1953. Heredity in health and mental disorder. 315 p., 89 figures, 20 tables. $6.00. W. W. Norton and Company, Inc., New York.

This is a general review of the author's studies of twins, especially as concerns mental disease but including also tuberculosis and certain other diseases, with an introductory section on human genetics and concluding discussion of public health applications, constituting the Salmon Memorial Lectures of 1952.

Leopold, Luna B., Editor, 1953. Round river. From the journals of Aldo Leopold. Illustrated by Charles W. Schwartz. 173 p. $3.00. Oxford University Press, New York.

Most of this book has been culled from journal notes of camping trips made in the twenties. They have, as Luna Leopold points out in his preface, "some of the pungency of oak smoke; the original pages are here and there spattered with a slapped mosquito or a drop of camp coffee. They convey some measure of insight into the camp and field experience from which perception gradually emerged." There are, in addition to the journal entries, a number of essays, found unfinished among Leopold's papers. These, finished or not, are notable contributions to the literature of conservation, fine examples of the clear thinking and clear prose of their author.

Light, S. F., 1954. Intertidal invertebrates of the Central California coast. 446 p. ill. $5.00. University of California Press, Berkeley, California.

A revision of Light's Laboratory and field text in invertebrate zoology by Ralph I. Smith, Frank A. Pitelka, Donald P. Abbott, Frances M. Weesner and others.

Mason, Stephen F., 1954. Main currents of scientific thought. 520 p. $5.00. Abelard-Schuman, Inc., New York.

Odum, Eugene, P., 1953. Fundamentals of ecology. 384 p., ill. $6.50. W. B. Saunders Company, Philadelphia.

Among the several recent manuals and texts of ecology this one is perhaps the most compact, best organized, and teachable. It covers a wide range of topics and approaches, except, unfortunately, the evolutionary approach, which, in the opinion of some ecologists, furnishes the most efficient integrative principle giving unity to the otherwise too diversified subject matter.

T. D.

Quastler, Henry, Editor, 1953. Information theory in biology. 273 p., ill. $4.00. University of Illinois Press, Urbana, Illinois.

Schrader, Franz, 1953 (2nd edition). Mitosis. 170 p., 19 Text-figures. $4.00. Columbia University Press, New York.

The second edition of this well-known work brings the reader up to date on the newer facts and ideas about the dynamics of mitosis. The principal new material considered is the extensive work of Östergren, which is carefully considered and critically evaluated in comparison with older ideas. Dr. Schrader emphasizes his well-founded opinion that mitosis can be explained only by assuming the activity of several different types of forces, and concludes that the mitosis problem will not be "revealed to us by a stroke of genius or luck" but "by some painstaking work that has to be guided by intelligent planning and a thorough knowledge of the results so far obtained." For the purpose of acquiring this knowledge and making the necessary plans, Dr. Schrader's book is indispensable.

Severinghaus, Aura E., Harry J. Carman, and William E. Cadbury, Jr., 1953.

Preparation for medical education in the liberal arts college. Report of the subcommittee on preprofessional education of the survey of medical education. 400 p., $4.50. McGraw-Hill Book Company, Inc., New York.

Simpson, George Gaylord, 1953. The major features of evolution. 434 p., 52 text figures. $7.50. Columbia University Press, New York.

This is a revised edition of the author's earlier classic "Tempo and Mode in Evolution," which in less than ten years has helped to produce its own obsolescence to such an extent that the new book has quite justifiably received a new title. Like its predecessor, the present book shows that the facts of paleontology are not only fully consistent with, but in fact yield strong support to, the modern theories of evolution developed primarily on the basis of genetics and of other disciplines dealing with the now living organisms. Some paleontologists and biologists, chiefly in Europe but also in the New World, have nevertheless interpreted paleontological findings as indicating the existence of evolutionary

forces unknown to or incompatible with genetics. These contentions are subjected to a thorough, tart, and altogether devastating critique. Those familiar, as well as those unfamiliar, with the "Tempo and Mode" have much to learn from "The Major Features of Evolution."

T. D.

Thayer, H. S., editor, 1953. Newton's philosophy of nature. Selections from his writings. Introduction by J. H. Randall, Jr. 208 p. $2.25, cloth; $1.15, paper. Hafner Library of Classics, No. 16. Hafner Publishing Company, New York.

Trevor, J. C., 1953. Race crossing in man, the analysis of metrical characters. 45 p., 1 figure, 24 tables. 12/6 or $2.50. Cambridge University Press, London and New York.

This is monograph 36 in the Eugenics Laboratory Memoirs of the Galton Laboratory, University College, London. A summary of selected data published by various authors up to 1938 concerning measurements of the body and its segments, especially the head, in groups deriving from matings between European and non-European racial groups, together with data on the parental groups, where available. A valuable summary table printed as an appendix, listing the standard durations of several dimensions in each hybrid group for comparison with variances of presumed parent groups is a striking reminder of the sparsity and scattering nature of information on the mensurable traits of human hybrids. The author, recognizing the limitations of the data, has restricted himself to critical reduction and ordering of the data and to the conclusion that "the available material does not suggest that the distributions of metrical characters in populations derived from the crossing of distinct racial groups are in any way peculiar when compared in populations of unmixed origin."

L. C. DUNN

Wood Jones, F., 1953. Trends of life. 191 p., $2.50. Edward Arnold and Company, London, St. Martin's Press, New York.

This is an impassioned protest of an old-school zoologist "against this tyranny of modern orthodox views" which fail to accept Lamarckism and teleology as reasonable explanations of evolution. The author is able to remain one of the few surviving psycho-Lamarckists because he feels "free to disregard the pathetic reiterations of the futile claims of those misguided experimentalists who have tried to interpret the ways of nature by taking upon themselves the role of the Divinity that shapes our ends." Nevertheless, the book ends in deep gloom, since the author believes that "the average intelligence of the masses is possibly on the decline," and fails to discern any escape from the altogether dismal trend of human evolution.

T. D.